Cognitive, biological and psychosocial factors predicting interferon-alpha-induced depression

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Abstract

Interferon-alpha (IFN-α) therapy for chronic hepatitis C virus (HCV) infection is associated with the development of depression and other neuropsychiatric adverse effects. However, well-defined predictors of this depression are still lacking. Several interlinked biological systems as well as cognitive and psychosocial factors may predispose individuals to the development of IFN-α-induced depression. The aim of this study was to identify such predictive factors as well as prospectively monitor the impact of IFN-α on a variety of clinical and biological outcomes.

Forty-eight patients with chronic HCV infection were recruited and assessed at baseline and after 4, 8, 12, 16, 20 and 24 weeks of IFN-α treatment. At each assessment, patients were evaluated with a number of questionnaires as well as the structured Mini International Neuropsychiatric Interview (MINI) for a diagnosis of major depressive disorder. Blood samples were also collected at all time points as well as salivary cortisol at baseline and end of treatment.

IFN-α-induced depression developed in 40% of the patients. Patients who developed IFN-α-induced depression had more negative illness perceptions, lower baseline levels of cortisol during the day, and lower baseline levels of kynurenic acid. Patients who developed IFN-α-induced depression also had altered gene expression in a number of pathways relevant for depression such as inflammation and neuroplasticity. Finally, detection and management of depression in this population is shown to be a complex process, reliant on the availability of clinical experts and good communication within a multidisciplinary team.

In conclusion, the findings of this study provide evidence for a number of cognitive, psychosocial and biological predictors of IFN-α-induced depression. These findings provide a rationale to test the effect of preventative cognitive interventions in these patients. However, future studies are needed to confirm some of these novel clinical and biological predictors, as well as to look at the interplay between these factors.

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List of abbreviations

AA	Arachidonic acid
ACTH	Adrenocorticotropic hormone
AIM2	Absent in melanoma 2
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
APA	American Psychiatric Association
AUC	Area under the curve
AUCi	Area under the curve of the increase
BDNF	Brain-derived neurotrophic factor
BLE	Brief Life Events
CAPSL	Calcyphosine-like
CECA	Childhood Experiences of Care and Abuse
CEQ	Cannabis Experience Questionnaire
CFQ	Chalder Fatigue Questionnaire
COX	Cyclooxygenase
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
CSF	Cerebrospinal fluid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, 4th Edition
DUX4L7	Double homeobox 4 like 7
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ERICH1	Glutamate-rich 1
FADS	Fatty acid desaturase

FKBP	FK506 binding protein
GBD	Global Burden of Disease
GDNF	Glial cell line-derived neurotrophic factor
GR	Glucocorticoid receptor
GSTM4	Glutathione S-transferase mu 4
HAAO	3-hydroxyanthranilate 3,4-dioxygenase
HADS-A	Hospital Anxiety and Depression Scale (anxiety subscale)
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPA	Hypothalamic-pituitary-adrenal
HPLC	High performance liquid chromatography
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
IDO	Indoleamine 2,3-dioxygenase
IDS	Inventory of Depressive Symptomatology
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IL	Interleukin
IL-1α	Interleukin-1-alpha
IL-1β	Interleukin-1-beta
IL-1R1	Interleukin-1 receptor, type 1
IL-6R	Interleukin-6 receptor
IL-28β	Interleukin-28-beta
IPQ	Illness Perceptions Questionnaire
JAK-STAT	Janus-activated kinase and signal transducers and activators of transcription
KAT	Kynurenine aminotransferase
KMO	Kynurenine 3-monooxygenase

Kpa	Kilopascals
KYN	Kynurenine
KYNA	Kynurenic acid
KYNU	Kynureninase
LA	Linoleic acid
LTP	Long term potentiation
MAPK	Mitogen-activated protein kinase
MCP-1	Human macrophage chemoattractant protein-1
MDD	Major depressive disorder
MINI	Mini International Neuropsychiatric Interview
MRC	Medical Research Council
mRNA	Messenger RNA
miRNA	Micro RNA
NBEAL	Neurobeachin-like1
NDM	Naturalistic decision making
ng	Nanogram
NF-κB	Nuclear factor kappa-light-chain-enhancer of B cells
nMol	Nanomolar
NMDA	N-methyl-D-aspartate
NR3C1	Nuclear receptor subfamily 3, group C, member 1
NT-3	Neurotrophin-3
OAS2	2’-5’-oligoadenylate synthetase 2
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
Peg-IFN-α	Pegylated interferon-alpha
PGE2	Prostaglandin E2
PIP	Phosphatidylinositol

PLA2	Phospholipase A2
PNPT1	Polyribonucleotide nucleotidyltransferase 1
PSS	Perceived Stress Scale
PUFAs	Polyunsaturated fatty acids
QUIN	Quinolinic acid
RNA	Ribonucleic acid
RNF144B	Ring finger protein 144B
sIL-2R	Soluble interleukin-2 receptor
sICAM-1	Soluble intracellular adhesion molecule-1
SF-36	Medical Outcomes Study Short-Form 36
SSRI	Selective serotonin reuptake inhibitor
SVR	Sustained virological response
TDO2	Tryptophan 2,3 dioxygenase
TGF-β1	Transforming growth factor beta-1
TNF-α	Tumor necrosis factor-alpha
TPH	Tryptophan hydroxylase 1
TRAF6	TNF receptor associated factor-6
TRP	Tryptophan
µg	Microgram
VEGF	Vascular endothelial growth factor A
VGF	non-acronymic
3-HK	3-hydroxykynurenine
5-HTTLPR	Serotonin transporter linked polymorphic region

1 Introduction

1.1 What is depression?

Major depressive disorder (MDD) is a highly prevalent neuropsychiatric condition characterised by a broad range of symptoms. The last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR), states five or more of a range of symptoms must be present, during a 2 week period for a diagnosis of MDD (APA, 2000). Furthermore, these symptoms must cause significant distress or impairment of normal functioning, and should not be attributable to a recent loss or be associated with a general medical condition or with substance abuse (APA, 2000). The symptoms for depression diagnoses are listed as:

- Depressed mood most of the day, nearly every day
- Diminished interest or pleasure in activities most of the day, nearly every day
- Significant weight loss or change in appetite nearly every day
- Insomnia or hypersomnia nearly every day
- Psychomotor agitation or retardation nearly every day
- Fatigue or loss of energy nearly every day
- Worthlessness, or excessive or inappropriate guilt nearly every day
- Diminished ability to think or concentrate, or indecision, nearly every day
- Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

MDD may manifest as a single episode or as recurrent episodes with a course of up to 2 years or longer in those with the single-episode form (Akiskal, 2009). The prognosis for recovery from an acute episode of MDD is good for most

patients, however, three out of four patients experience recurrences throughout life with varying degrees of residual symptoms between episodes (Akiskal, 2009). Lifetime prevalence of MDD varies widely across different populations with the majority being in the range of 8-12% (Andrade et al., 2003). The incidence of MDD has and continues to steadily increase, and the mean age of onset has been reported to have decreased to around 27 years old (Kessler, 2002). One of the most consistent socio-demographic correlates of MDD across populations is being female (Andrade et al., 2003, Eaton et al., 1997, Kendler et al., 1993). MDD has a multifactorial aetiology originating from the interaction between environmental and genetic factors, and presents frequent comorbidity (Zunszain et al., 2012b).

1.2 Inflammation and Depression

1.2.1 Introduction

Since MDD is a complex disorder, it is likely that alterations in several interacting systems underlie its pathogenesis. As such, numerous hypotheses have been proposed to elucidate its origins. One of these is the inflammatory hypothesis, initially suggested as the macrophage theory of depression (Smith, 1991), and now also known as the malaise or cytokine theory of depression (Maes et al., 2009, Miller et al., 2009). This particular hypothesis emphasises the role of psycho-neuroimmunological dysfunctions where there is an activation of the immune system. Subsets of MDD patients, who are otherwise medically healthy, have been repeatedly shown to have an altered peripheral immune system. This includes impaired cellular immunity, increased levels of pro-inflammatory cytokines, increased levels of acute phase proteins, and increased expression of chemokines and adhesion molecules (Bouhuys et al., 2004, Maes, 1995, Miller et al., 2002, Musselman et al., 2001b). Studies have indicated that innate immune cytokines can influence pathophysiological domains, such as neurotransmitter metabolism, neuroendocrine function and regional brain activity, all of which are relevant to MDD (Dantzer et al., 2008, Schiepers et al., 2005). Indeed, administration of high levels of pro-inflammatory cytokines has been shown to cause changes in behaviour, such as low mood, fatigue, anxiety, sleep disturbances, anhedonia and cognitive dysfunction, all of which closely resemble symptoms observed in MDD (Capuron and Miller, 2004, Pollak and Yirmiya, 2002), and which constitute the main focus of my PhD.

1.2.2 Cytokine abnormalities in depression

Several biomarkers of inflammation have been measured in MDD and some of the most frequently reported are cytokines including interleukin-1 (IL-1), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α). Also of importance is C-reactive protein (CRP) which is produced by hepatocytes in response to IL-6. Recent meta-analyses have reported positive associations of all of these immune molecules with MDD, in both serum and plasma (Dowlati et al., 2010, Howren et al., 2009). Moreover, some studies have demonstrated these elevations occur not only in peripheral blood, but also in the central nervous system, in particular in cerebrospinal fluid (CSF) (Levine et al., 1999). Additionally, some of these increases have also been measured in the brain, with post-mortem gene expression analyses showing an up-regulation of a variety of pro- and anti-inflammatory cytokines in the prefrontal cortex of MDD patients (Shelton et al., 2011). Similarly, mRNA and protein expression levels of IL-1β, IL-6, and TNF-α have been shown to be significantly increased in the brains of teenage suicide victims when compared with normal control subjects (Pandey et al., 2011). Depressed patients have also been shown to have higher levels of other acute phase proteins, chemokines and cellular adhesion molecules including a-1-acid glycoprotein, a-1-antichymotrypsin, haptoglobin, human macrophage chemoattractant protein-1 (MCP-1), soluble intracellular adhesion molecule-1 (sICAM-1) and E-selectin (Raison et al., 2006).

It is still unclear whether or not these changes occur before and therefore lead to the development of MDD, or are instead a consequence of the illness. That is, is an altered immune system an ‘‘at-risk’’ or a ‘‘state’’ condition? For a long time the brain was considered, from an immunological point of view, to be a

privileged organ. It is now known that pro-inflammatory cytokines are produced in response to insults not only by immune cells but also by neurons and neural stem cells (Tsakiri et al., 2008, Zunszain et al., 2012a). Cytokines can act directly on the brain causing changes known as ‘‘sickness behaviour’’, a co-ordinated set of subjective, behavioural and physiological changes that develop in sick individuals during the course of an infection (Dantzer, 2004). If there is a prolonged activation of the peripheral immune system, the immune signalling to the brain can lead to an exacerbation of sickness behaviour and to the development of depressive symptoms in vulnerable individuals (Dantzer et al., 2008). The administration of cytokines and several animal models, discussed below, support this view. Further support for an aetiological role of cytokines in MDD comes from longitudinal studies. For example, a decade-long study found that serum levels of high sensitivity CRP in women is an independent risk marker for de novo MDD (Pasco et al., 2010). Similarly, in a 12-year follow-up study, levels of CRP and IL-6 have been shown to predict the subsequent development of MDD (Gimeno et al., 2009).

1.2.3 Immune stimulation and “sickness behaviour”

As mentioned above, pro-inflammatory cytokines can induce behavioural symptoms referred to as sickness behaviour (Dantzer, 2001b). Acute immune stimulation via the administration of pro-inflammatory cytokines or by treatment with cytokine-inducers has been shown to lead to emotional symptoms indicative of sickness behaviour and depression. For example, a single exposure to *Salmonella typhi* vaccine has been shown to increase negative mood in conjunction with increases in IL-6 (Wright et al., 2005). In addition to a robust inflammatory response as indicated by increased levels of IL-6,

Salmonella typhi vaccine has been demonstrated to cause increases in subjective ratings of fatigue and confusion, as well as evoke neural activity within the substantia nigra during a cognitive task (Brydon et al., 2008). In two further studies it was shown that *Salmonella typhi* but not placebo, causes inflammation-related mood reduction, accompanied by enhanced activity in several brain regions including: subgenual anterior cingulate cortex (sACC), thalamus, amygdale, cingulate and anterior insula (Harrison et al., 2009a, Harrison et al., 2009b). Using *Salmonella abortus equi* endotoxin, increases in levels of anxiety and depressed mood, together with a decrease in verbal and non-verbal memory functions has also been shown (Reichenberg et al., 2001). Furthermore, these changes correlated with increased circulating levels of TNF-α, soluble TNF receptors, IL-6, IL-1 receptor antagonist and cortisol. Feelings of social disconnection and depressed mood, together with significant increases in IL-6 and TNF-α have also been observed after a single exposure to *Escherichia coli* endotoxin (Eisenberger et al., 2010b). Moreover, short-term exposure to low-dose *Escherichia coli* endotoxin has been shown to be associated with increased depressed mood over time in healthy individuals, by altering reward-related neural responses (Eisenberger et al., 2010a).

Animal studies have also provided useful evidence for a putative role of cytokines in the context of MDD. Administration of different pro-inflammatory cytokines in rodents has been shown to result in sickness symptoms including: decreased interest in exploration, depressed motor activity, withdrawal from social activities, reduced food and water intake, deficient cognition, hyperalgesia and hypersomnia (Dantzer, 2001a, Yirmiya et al., 2002). In a more indirect way, mice subjected to chronic mild stress show increased IL-1β levels

in the hippocampus and in parallel, changes in behaviour resembling depressive symptoms, such as decreased sucrose preference and reduced social exploration. In contrast, mice with a deletion of the IL-1 receptor do not display such behavioural changes (Goshen et al., 2008). In another indirect study, constant darkness produced depression-like behaviour in mice with concomitant elevated levels of IL-6 in plasma as well as in the hippocampus (Monje et al., 2011). Furthermore, exposure to TNF-α has also been shown to produce depressive-like states in mice, an effect which can then be blocked by co-administration of an anti-TNF-α antibody (Kaster et al., 2012). Administration of cytokine antagonists, such as IL-1 receptor antagonist, or anti-inflammatory cytokines such as IL-10, can block the behavioural effects of treatment with cytokines and/or endotoxins in animals (Bluthe et al., 1995, Dantzer et al., 2008, Kent et al., 1992). Interestingly, treatment of animals with antidepressants can also relieve some of the symptoms of cytokine-induced sickness behaviour (Pollak and Yirmiya, 2002).

1.2.4 Other evidence supporting a role for inflammation in depression

Two further series of evidence support a role for inflammation in depression. Firstly, it is well documented that depression occurs 5 to 10 times more often in the medically ill than in the general population and has a significant impact on quality of life, treatment adherence, morbidity, and mortality (Evans et al., 1999). Furthermore, MDD is particularly common in conditions with an inflammatory component, such as cardiovascular disease and rheumatoid arthritis, as well as in autoimmune and neurodegenerative disorders (Evans et al., 2005, Pollak and Yirmiya, 2002, Wise and Taylor, 1990).

Secondly, the contribution of psychosocial stress to the development of MDD is also well-documented (Mundt et al., 2000) and this may be mediated by an activation of inflammatory processes. Both acute and chronic psychosocial stress have been shown to activate innate immune signalling pathways as well as innate immune cytokines such as IL-6 (Bierhaus et al., 2003). For example, chronic stress including difficult caregiving and hostile marital relationships, has been associated with increased levels of CRP (Miller, 2008). Early life stress may be especially relevant as it appears to produce neuroendocrine and immunological abnormalities that are thought to mediate the development of a pro-inflammatory phenotype in adulthood (Chida et al., 2007, Elenkov, 2008, Hepgul et al., 2012). Several studies have demonstrated that childhood trauma predisposes to increased inflammation in adulthood, as shown by higher levels of CRP and fibrinogen, as well as by increased reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Danese et al., 2008, Heim et al., 2008). In healthy individuals, childhood maltreatment has been associated with increased levels of CRP in adulthood, independent of other co-occurring early-life risks, current stress or health problems (Danese et al., 2009, Danese et al., 2007). Similarly, exposure to an acute stressor, in men with MDD and a history of early life stress, has been shown to cause an exaggerated IL-6 response, together with increased DNA binding of the key pro-inflammatory transcription factor, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in peripheral blood mononuclear cells (Pace et al., 2006). Additionally, greater acute IL-6 release and higher IL-6 concentrations over time have been observed in individuals with moderate to severe childhood maltreatment (Carpenter et al., 2010).

1.3 Interferon-alpha for Hepatitis C infection: a clinical model of cytokine-induced depression

1.3.1 Introduction

In order to investigate the pathways through which inflammation and cytokines can induce depression, it is essential to have relevant model systems. One such model system involves patients undergoing treatment with the pro-inflammatory cytokine interferon-alpha (IFN-α). This is an important and widely used model as it is an existing clinical observation that a high percentage of patients who are administered IFN-α develop a behavioural syndrome that is strikingly similar to major depression. As such, this model can be readily utilised for observational studies without the need for any interventional measures.

1.3.2 Hepatitis C infection

Since the isolation of its pathogenic agent, the Hepatitis C Virus (HCV), in 1989, hepatitis C has grown as a public health concern. HCV infection affects around 200 thousand people in England, and approximately 170 million worldwide (See Figure 1.1) (Harris et al., 2011, Lavanchy, 2009). There are various routes of transmission of HCV and up until the 1980s, one of the most common was exposure to contaminated blood or blood products. However, since the introduction of routine testing of donated bloods, transmission via blood transfusion has been virtually eliminated (Donahue et al., 1992). Today, the use of injectable drugs is the main source of HCV infection in most developed countries and is also an increasing source of infection in developing countries (Palmateer et al., 2012, Sweeting et al., 2009, Wasley and Alter, 2000). Furthermore, in developing countries, the re-use of contaminated or inadequately sterilized equipment in medical and dental procedures is another

major source of transmission (Hutin and Chen, 1999, Ver Hoeve et al., 2012). Generally, acute HCV infection is relatively asymptomatic because it replicates slowly and so the infection may take decades to progress. However, it is estimated that up to 85% of patients with acute HCV will develop chronic HCV infection, and nearly 20% will develop liver cirrhosis (Fattovich et al., 1997, GBD, 2004, Sockalingam and Abbey, 2009). In fact, HCV infection is the leading cause of cirrhosis and the main indication for liver transplantation worldwide (Schiff, 2011). As a result of cirrhosis, 1-5% of patients may also go on to develop hepatocellular carcinoma (GBD, 2004). Complicating the picture further, HCV exists in at least six genotypes and treatment success varies by genotype (see below).

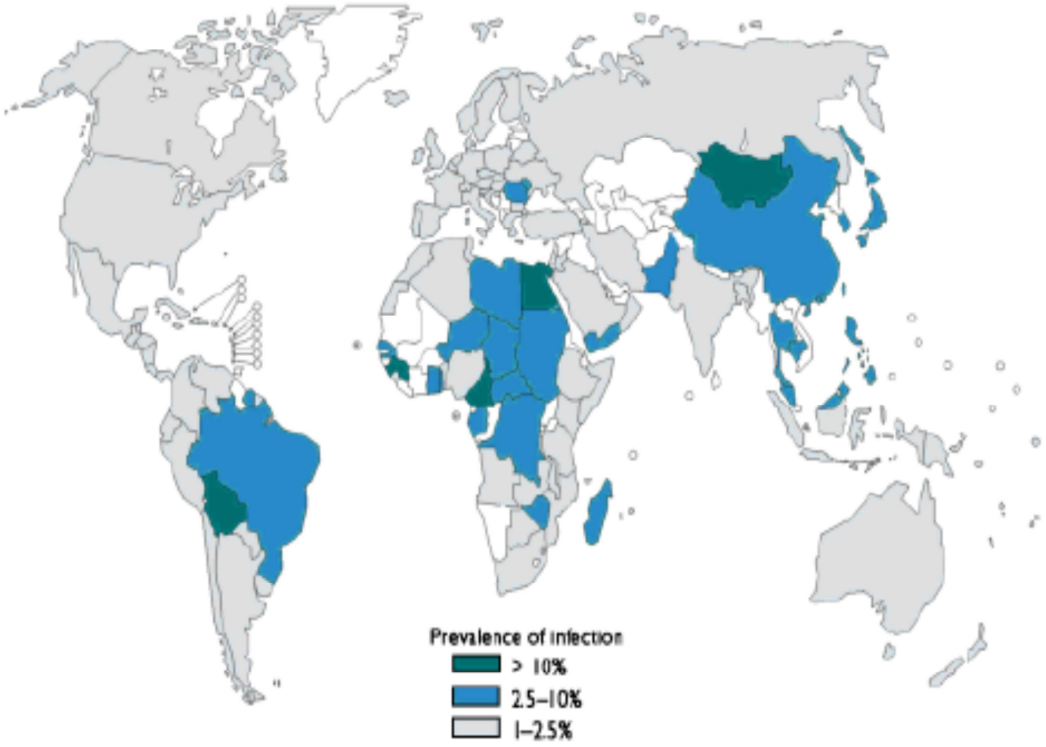


Figure 1.1 The global prevalence of Hepatitis C

The estimated global prevalence rates of Hepatitis C infection in the year 2004. Figure from (Lavanchy, 2009): The Global burden of hepatitis C. *Liver International, 29,* 78-81.

1.3.3 Interferon-alpha for Hepatitis C infection

Standard treatment for HCV infection involves a combination of once-weekly subcutaneous pegylated-interferon-alpha (peg-IFN-α) and twice-daily oral ribavirin; an antiviral agent. This treatment is usually given for 24 weeks (for genotypes 2 and 3) or for 48 weeks (for genotypes 1, 4 and 5) (Sockalingam and Abbey, 2009). IFN-α is a cytokine released by the innate immune system and has an important function in the innate antiviral response (Feld and Hoofnagle, 2005). IFN-α can attach to cell-surface receptors that signal through Janus-activated kinase (JAK) and signal transducers and activators of transcription, leading to induction of multiple IFN-stimulated genes (Hoofnagle and Seeff, 2006). These genes include double-stranded RNases, viral protein translation inhibitors, and proteins which destabilize viral messenger RNA. IFN-α also induces the expression of genes involved in the innate immune response and acutely induces the production and release of other innate immune cytokines such as IL-6 and TNF-α (Raison et al., 2008). As well as this, IFN-α is also thought to facilitate the recognition of virus-infected or tumour cells by cytolytic T-lymphocytes (Wichers and Maes, 2002). As such, IFN-α possesses marked anti-viral, anti-cancer, and immunomodulatory properties, and is also approved for the treatment of a number of other disorders including: multiple sclerosis, malignant melanoma and chronic myelogenous leukaemia (Piper et al., 2001).

For HCV infection, response to IFN-α treatment is measured by Sustained Virological Response (SVR) rates, defined as undetectable HCV levels 6 months post treatment. The overall rate of SVR was generally low with IFN-α monotherapy and the subsequent addition of ribavirin led to improvements in

SVR rates. Ribavirin is a nucleoside analogue with broad activity against viral pathogens (Feld and Hoofnagle, 2005). The mechanisms of action of ribavirin against HCV are not completely clear, however, it appears to have immunomodulatory effects as well as modulating the expression of IFN-stimulated genes and inducing viral mutagenesis (Paeshuyse et al., 2011). With this combination therapy, SVR rates approach 50% for genotype 1 and about 80% for genotypes 2 and 3 (Agarwal et al., 2007).

Two new antiviral products have recently been licensed for the treatment of HCV infection in combination with IFN-α and ribavirin. Bocepravir and telaprevir are both protease inhibitors which have been approved for use in treatment of HCV genotype 1 (Ghany et al., 2011). Phase III trials have shown that in combination with the standard treatment, both boceprevir and telaprevir increase SVR rates for genotype 1 from less than 50% to 70%, while also cutting treatment time in half for some individuals (Gravitz, 2011). However, as these drugs will supplement and not replace the standard IFN-α plus ribavirin therapy, they will not eliminate the difficult side effects associated with HCV treatment. Despite its efficacy on improving SVR rates, unfortunately IFN-α induces a number of neurotoxic effects such as depression, anxiety, mania and fatigue (Sockalingam and Abbey, 2009).

1.3.4 IFN-α-induced neuropsychiatric effects

After the initial injection of IFN-α, almost all patients experience an acute cytokine-induced sickness behaviour, including malaise, myalgia, anorexia, fatigue, apathy, poor concentration and attention, non-specific pain and several other flu-like symptoms (Capuron and Miller, 2004, Dieperink et al., 2000,

Raison et al., 2005b). Flu-like symptoms such as fever, cough, dyspnea, pharyngitis, rhinorrhea and rash generally subside in 1-2 weeks, however, fatigue, malaise, apathy and cognitive and behavioral changes usually persist for several weeks throughout the treatment period. This sickness behaviour induced by IFN-α is consistent with the effects seen with cytokine administration in animals as mentioned earlier (Dantzer, 2004, Konsman et al., 2002, Raison et al., 2006, Yirmiya et al., 2002).

1.3.5 Clinical features of Interferon-alpha-induced depression

The symptoms caused by IFN-α are similar to that of somatic or vegetative symptoms seen in MDD (Raison and Miller, 2003). Table 1.1 demonstrates the symptoms of acute sickness behaviour induced by IFN-α therapy, which overlap with and are commonly manifested symptoms seen in MDD patients. The development of MDD during IFN-α therapy in patients with HCV infection is common with an incidence of up to 45% (Asnis and De La Garza, 2006, Capuron and Miller, 2004, Raison et al., 2005b).

Table 1.1 Similarities between MDD and sickness behaviour

	Depression	**Sickness Behaviour**
Somatic Effects	Fatigue/loss of energy	Fatigue
	Weight loss/gain	Weight loss
	Appetite loss/increase	Anorexia
	Insomnia/hypersomnia	Sleep disorders
		Depressed mood
		Behavioural despair
Behavioural Effects	Depressed mood	Social withdrawal
	Worthlessness/guilt	Anhedonia
	Loss of interest	Cognitive impairment
	Anhedonia	Suppression of motor behaviour
	Inability to concentrate	
	Psychomotor agitation/retardation	
	Suicide ideation	

Adapted from (Smith et al., 2011b): Risk factors for the development of depression in patients with hepatitis C taking interferon-α. *Neuropsychiatric Disease and Treatment*, *7,* 275-92.

The onset of depressive symptoms usually occurs within the first 3 months of IFN-α therapy (Capuron et al., 2002b). Interestingly, dimensional analyses of IFN-α-induced symptoms have shown evidence for the development of two behavioural syndromes; a neurovegetative versus a mood and cognitive syndrome (See Figure 1.2) (Capuron et al., 2002a, Capuron and Miller, 2011). The neurovegetative syndrome develops rapidly in almost all patients and persists for the duration of IFN-α treatment. This consists of symptoms of fatigue, psychomotor slowing, pain, loss of appetite and sleep disturbances. On the other hand, the mood and cognitive syndrome, characterized by low mood, anxiety, as well as memory and attention deficits, usually appears later on in treatment (Capuron et al., 2002a, Capuron and Miller, 2004). Furthermore, the mood and cognitive symptoms appear to respond to treatment with antidepressants whereas the neurovegetative symptoms do not (Capuron and Miller, 2004, Capuron and Miller, 2011). These two distinct syndromes may have different underlying pathophysiology and as such require different treatment strategies (Capuron and Miller, 2004).

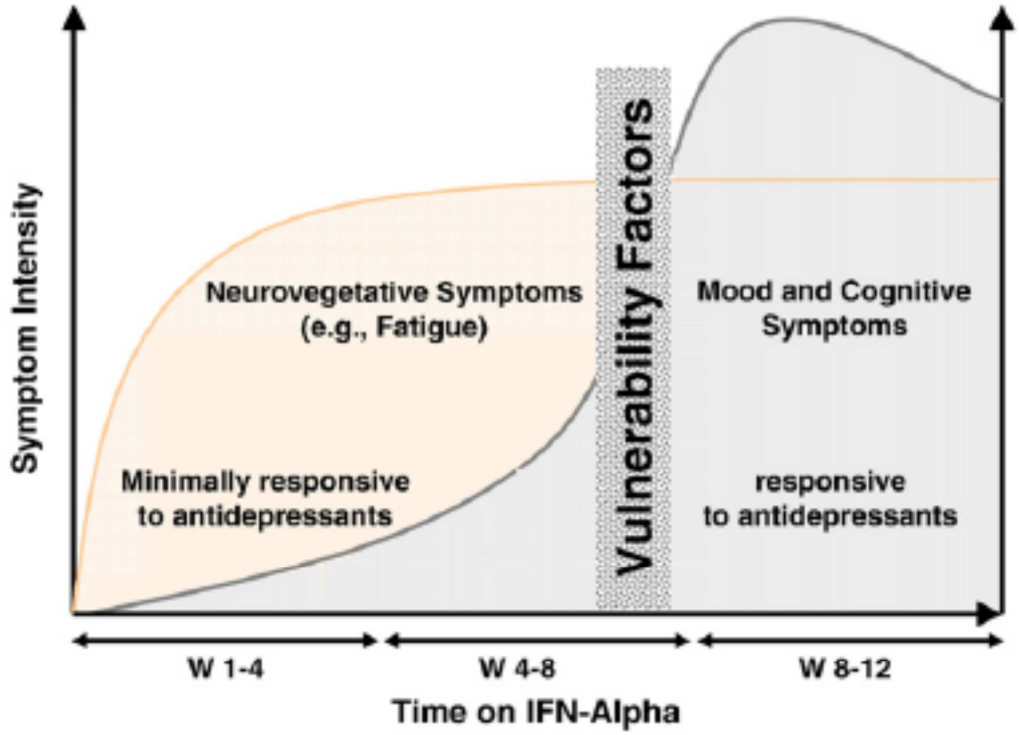


Figure 1.2 The development of two behavioural syndromes; a neurovegetative versus a mood and cognitive, over the course of IFN-α treatment

IFN-α treatment induces two types of behavioural symptoms both with a different time course and antidepressant response. Neurovegetative symptoms develop rapidly, whereas mood and cognitive symptoms develop at a later stage. Figure from (Capuron and Miller, 2011): Immune system to brain signaling: Neuropsychopharmacological implications. *Pharmacology & Therapeutics, 130*, 226-38.

Furthermore, the experience of depressive symptoms during the course of antiviral treatment has important negative consequences, such as impairing quality of life, reducing compliance as well as leading to dose reduction or discontinuation of treatment, all of which compromise the therapeutic response to the treatment (Asnis and De La Garza, 2006). In some rare cases IFN-α-induced depression can be so severe that patients experience suicidal ideation (Ademmer et al., 2001, Dieperink et al., 2004), and a few reports describe patients who have attempted or committed suicide (Fukunishi et al., 1998, Janssen et al., 1994, Sockalingam et al., 2011). As such, effective and timely detection of IFN-α-induced depression is important to ensure that appropriate treatment and support can be provided.

1.3.6 Clinical predictors of IFN-α-induced depression

1.3.6.1 Previous history and baseline levels of depression

There is conflicting evidence about what factors are predictive of depressive symptoms during IFN-α treatment. One clinical predictor most often associated with subsequent development of depression during treatment is the presence of mood or anxiety symptoms prior to treatment. Studies have suggested that a previous history of psychiatric illness, especially MDD, increases vulnerability to the development of depression on treatment (Castera et al., 2006, Raison et al., 2005a). Furthermore, higher baseline depression scores have been consistently demonstrated to predict later scores and subsequent development of MDD during treatment (Castellvi et al., 2009, Dieperink et al., 2003, Evon et al., 2009, Lotrich et al., 2007).

It has also been reported that the development of depressive and neuropsychiatric symptoms in the early stages of therapy can predict the later development of MDD (Capuron et al., 2002b). Family history of a mood disorder or the presence of a sleep disorder has also been suggested to predict the occurrence of IFN-α-induced neuropsychiatric symptoms (Raison et al., 2005b, Sockalingam and Abbey, 2009). It could, however be argued that pre-existing vulnerability as a risk factor has such support as it has been the most extensively investigated, and in fact a history of a psychiatric disorder before starting IFN-α therapy does not unequivocally predict the occurrence of such symptoms (Pariante et al., 1999). In fact, it has been previously demonstrated that after adjusting for baseline scores, patients with a pre-existing psychiatric diagnosis and patients with no psychiatric history had no difference in maximal depression or anxiety scores (Pariante et al., 2002). Furthermore, the same study found no significant difference between groups in the incidence of adverse psychiatric effects severe enough to require psychopharmacological treatment. This is an important issue as in some cases a previous history of psychopathology has been considered as a significant cause for treatment withdrawal, due to the theoretical risk of worsening of psychiatric symptomatology induced by IFN-α treatment. Withholding IFN-α in this way, especially from members of a stigmatized class, raises questions about fairness and discrimination (Spennati and Pariante, 2012). In my PhD, I will assess the difference between using absolute values versus change from baseline scores in order to have a clearer understanding of the impact of a previous history of depression.

1.3.6.2 Other predictors

Some studies have investigated demographic and social risk factors; however, there appears to be no consensus on whether or not these impact on the development of depression (Raison et al., 2005a, Smith et al., 2011b). One risk factor which appears to be related to depressive outcomes is that of social support. Indeed, lower levels of social support have been shown to significantly increase the risk of developing depression during IFN-α therapy (Evon et al., 2011, Evon et al., 2009). It is interesting to note the absence, among the clinical predictors that have been investigated, of a “usual suspect” risk factor for the development of depression, especially in relationship with stress and inflammation - a history of childhood trauma. As mentioned previously there is clear evidence, across different populations, that childhood trauma predisposes to inflammation in adulthood as well as depression (Archer et al., 2012, Caspi et al., 2003, Danese and McEwen, 2012, Danese et al., 2009, Danese et al., 2008).

Few studies have evaluated the link between depression and patients’ health related quality of life during therapy, which can be influenced not only by the biological and symptomatic aspects of the therapy but also by a person’s perceptions of their health status (Hunt et al., 1997). Illness perceptions are a patient’s own implicit common sense beliefs about their illness, and the manner in which patients perceive their illness and subsequent therapy is likely to influence many aspects of their experience including side-effects (such as developing depression) and other health outcomes. Across disease groups, and particularly in chronic disorders, illness perceptions have been found to be associated with several emotional well-being and quality of life outcomes

(Endermann and Zimmermann, 2009) however, they have not previously been investigated in patients receiving IFN-α therapy for HCV infection.

1.3.7 Importance of identifying predictive factors

It is important to identify predictors of IFN-α-induced depression in order to understand which individuals may require an intervention, particularly treatment with antidepressants. Prophylactic treatment with antidepressants in the form of selective serotonin reuptake inhibitors (SSRIs) has been shown to be effective in preventing IFN-α-induced depression in patients with malignant melanoma (Musselman et al., 2001b). However, attempts of using antidepressants prophylactically in patients with chronic HCV infection have shown less promising results. Only eight randomised placebo-controlled trials of prophylactic antidepressant treatment exist, and the majority of these studies suggest it is not always effective without any major differences seen between placebo and antidepressants (Diez-Quevedo et al., 2010, Morasco et al., 2010, Morasco et al., 2007, Musselman et al., 2001a, Raison et al., 2007).

Moreover, some studies suggest extreme caution in the use of SSRIs in this condition as SSRIs have antithrombotic action that further increases the risk of haemorrhages in these patients, in the presence of IFN-α-induced thrombocytopenia and oesophageal varices (Weinrieb et al., 2003). As well as this, patients receiving IFN-α are at risk of developing mania (Onyike et al., 2004, Quelhas and Lopes, 2009), a risk which is notably increased by antidepressants (Beckwith, 2008, Wu et al., 2007). Furthermore, the altered liver function found in these patients could change the metabolism of antidepressants, with additional toxicity risks associated with potentially higher

plasma concentrations of these drugs. Finally, many patients are reluctant to take psychoactive medication, particularly given an often prolonged history of drug abuse. Other treatment options include psychiatric interventions which have been suggested to reduce depressive symptoms (Farber et al., 2005, Neri et al., 2010) and cognitive behavioural therapy which has been successfully used to prevent the development of depression during treatment (Ramsey et al., 2011). Given that prophylactic treatment “for all” is not a feasible option; it is highly important to better understand the underlying mechanisms by which IFN-α-induced depression develops.

1.3.8 Mechanisms of IFN-α-induced depression

Identifying biological predictors of IFN-α-induced depression is not only clinically useful but can also help in defining the molecular mechanisms of cytokine-induced depression. Previous works by my supervisors and others have identified genetic markers on the serotonin transporter and IL-6 genes (Bull et al., 2009, Kraus et al., 2007), as well as in genes involved in the metabolism of polyunsaturated fatty acids (Su et al., 2010), which seem to predict the development of IFN-α-induced depression. As discussed below, other studies have examined changes in biomarkers such as cortisol (Capuron et al., 2003b), serum tryptophan concentrations (Capuron et al., 2003a) and even brain functions (Capuron et al., 2005, Taylor et al., 2013) during IFN-α treatment.

1.3.8.1 HPA axis

A disturbance in HPA axis functionality is a well-established and consistent finding in MDD (Miller et al., 2009, Pariante, 2003, Pariante, 2006, Stetler and Miller, 2011). Regulation of the HPA axis starts by release of corticotropin-

releasing hormone (CRH) from the paraventricular nucleus of the hypothalamus. CRH leads to the release of adrenocorticotropic hormone (ACTH) in the pituitary, inducing discharge of cortisol from the adrenal cortex. In healthy individuals, cortisol exerts a negative feedback mechanism that controls its production, by regulating the synthesis of CRH (See Figure 1.3).

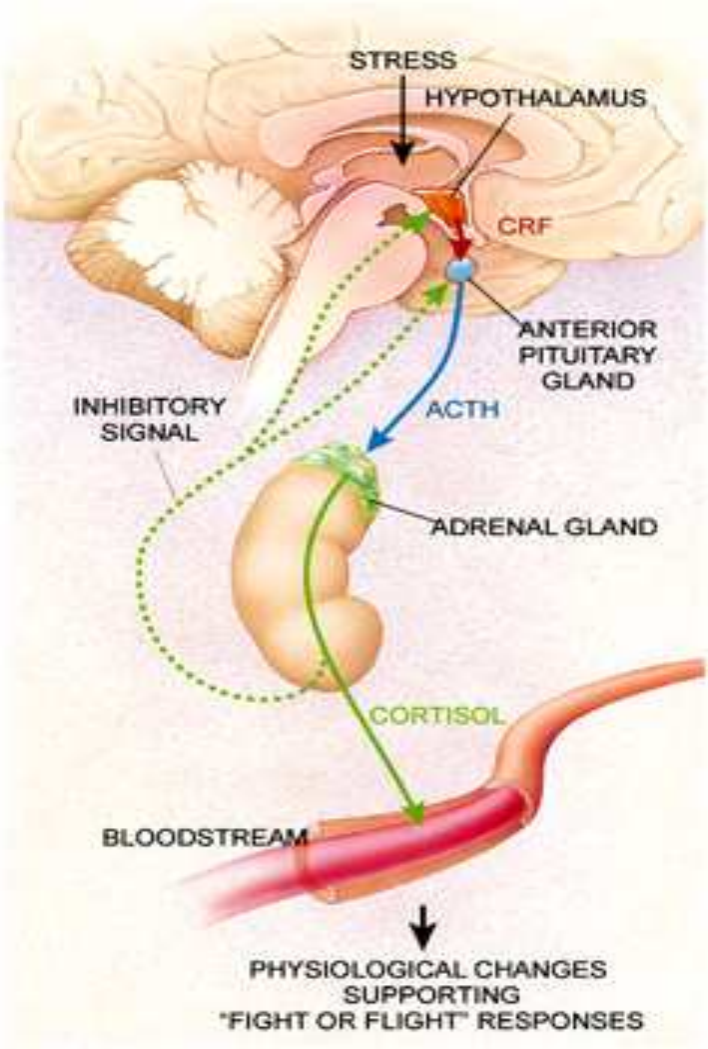
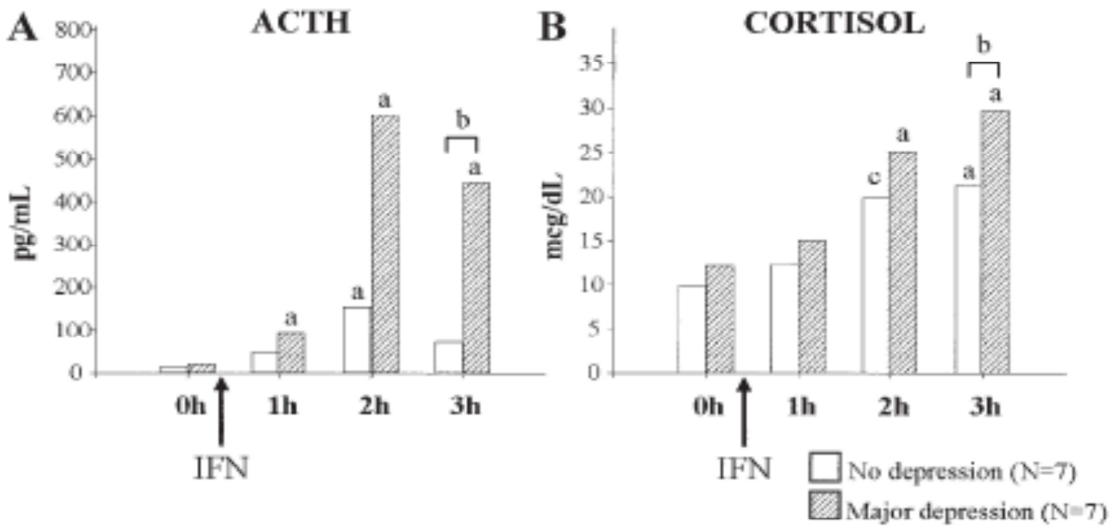


Figure 1.3 The hypothalamus-pituitary-adrenal (HPA) axis

The hypothalamus-pituitary-adrenal (HPA axis) activity is mediated by the secretion of corticotrophin releasing factor (CRH) from the hypothalamus, which in turn activates the secretion of adrenocorticotropin hormone (ACTH) from the pituitary, which finally stimulates the secretion of cortisol from the adrenal cortex. Cortisol interacts with its receptors in multiple target tissues including the HPA axis, where it is responsible for feedback inhibition of ACTH and CRH. Figure from (Nemeroff, 1998): The neurobiology of depression. *Scientific American, 278,* 42-9.

Interferons are known to activate the HPA axis (Dafny and Yang, 2005) and so this pathway may also be involved in the development of IFN-α-induced depression. Indeed, administration of cytokines has been shown to stimulate the expression and release of CRH, ACTH and cortisol, all of which have been found to be elevated in MDD (Pace et al., 2007, Pariante and Miller, 2001). Few studies have assessed the relationship between IFN-α and HPA axis activity, and these have produced mixed results.

Acute activation of the HPA axis after administration of IFN-α has been shown to be associated with subsequent development of depressive symptoms, with an exaggerated cortisol response to the first injection of IFN-α predicting the future occurrence of depression (Capuron and Miller, 2004). As shown in Figure 1.4, both ACTH and cortisol levels are higher in patients who later go on to develop IFN-α-induced depression and these effects are evident within 2 hours of IFN-α administration. This suggests that a “hyper-reactive” HPA axis is a risk factor for developing IFN-α-induced depression. Interestingly, the same study showed that these initial ACTH and cortisol responses correlated with depressive, anxiety and cognitive symptoms, but not with neurovegetative or somatic symptoms. This supports the notion that distinct symptom dimensions induced by IFN-α, may have different underlying mechanisms (Capuron and Miller, 2004, Capuron and Miller, 2011).



C

Correlations

Symptom Dimension	ACTH	CORTISOL
Depression	.67**	.53*
Anxiety	.70**	.52*
Cognitive Dysfunction	.66*	.66**
Neurovegetative	.35	.40
Somatic	.29	.24

Figure 1.4 ACTH and cortisol response to the first injection of IFN-α, and the association with the future occurrence of depression

Adrenocorticotropic hormone (ACTH) **(A)** and cortisol **(B)** responses to the first injection of IFN-α are higher in patients who subsequently develop IFN-α-induced depression, and correlate with mood and cognitive symptoms but not neurovegetative or somatic symptoms **(C)**. Figure from (Capuron and Miller, 2004): Cytokines and Psychopathology: Lessons from Interferon-α. *Biological Psychiatry, 56,* 819-24.

However, data from studies looking at chronic administration have been less reliable. In one study, IFN-α has been shown to induce a progressive increase in cortisol output during the day, accompanied by a reduction in the cortisol awakening response, both becoming significant after 8 weeks of treatment (Wichers et al., 2007). A second study also found IFN-α-induced depression to be associated with increased evening cortisol levels, and a consequent flattening of the cortisol rhythm (that is, a smaller difference between the morning peak and the evening through), an abnormality also described in MDD (Raison et al., 2008). On the other hand, some studies have found that IFN-α does not induce changes in plasma cortisol levels and cannot be correlated with depression scores (Fontana et al., 2008). Wichers et al. reported no association between daily average salivary cortisol concentrations or the cortisol awakening response and depressive symptoms in HCV patients undergoing IFN-α treatment (Wichers et al., 2007).

One pathway by which cytokines may influence HPA axis function is through their effects on negative feedback regulation which itself is thought to be mediated, at least in part, by alterations in the glucocorticoid receptor (GR) (Miller et al., 2009). Cytokine activation of signalling pathways, such as p38 mitogen-activated protein kinase (MAPK), and NF-κB have been shown to inhibit GR function and decrease GR expression (Dantzer et al., 2008, Pace et al., 2007). Indeed, it was recently demonstrated that following the initial injection of IFN-α, an activation of p38 MAPK in peripheral blood lymphocytes, was associated with subsequent depression and fatigue. This suggests that increased sensitivity of p38 MAPK signalling pathways may represent a vulnerability to IFN-α-induced depression (Felger et al., 2011). In my PhD, I will

assess the relationship between inflammation and HPA axis activity by measuring salivary cortisol. I will investigate whether HPA axis alterations are evident at baseline, and if these are associated with subsequent development of IFN-α-induced depression. I will also examine the effect of IFN-α on salivary cortisol levels over the course of the treatment period.

1.3.8.2 Serotonin system

There is a wealth of evidence to suggest that the neurotransmitter serotonin plays a role in MDD (Owens and Nemeroff, 1994) and it is suggested that IFN-α also has effects on the function of the serotonin system (Schaefer et al., 2003). Indeed, polymorphisms in the serotonin transporter linked polymorphic region (5-HTTLPR) have been shown to influence the development of IFN-α-induced depression. Specifically, two studies have found evidence for an “at risk” effect of S/S genotype and a “protective” effect of L/L genotype in the development of IFN-α-induced depression (Bull et al., 2009, Lotrich et al., 2009). More recently, a third study also investigated the same polymorphic region and found no statistically significant differences between S/S and L/L genotypes in either depression or anxiety levels, however subjects with L/L genotype did present lower changes from baseline for depressive symptoms (Udina et al., 2013). The involvement of the serotonin system in the development of IFN-α-induced depression is further supported by the ability of SSRIs to successfully ameliorate depressive symptoms (Gupta et al., 2006, Kraus et al., 2002). In fact, a recent literature review, demonstrated that most studies report SSRIs to have a very high success rate in treating IFN-α-induced depression – up to 85% (Baraldi et al., 2012). Furthermore, this is well beyond the treatment outcomes observed in randomized controlled clinical trials in major depression outside of

the context of IFN-α therapy, where the overall efficacy for most old and new antidepressant treatments is never higher than 50% (Khan et al., 2005, Parker, 2005).

Many investigations of the serotonergic system have focused specifically on the role of the enzyme indoleamine 2,3- dioxygenase (IDO) which can be induced by several pro-inflammatory cytokines (Wirleitner et al., 2003). When activated, IDO breaks down the essential amino acid tryptophan, the primary precursor of serotonin, into kynurenine (KYN) (Schwarcz and Pellicciari, 2002) (See Figure 1.5). KYN is a precursor of the bioactive metabolites quinolinic acid (QUIN) and kynurenic acid (KYNA). QUIN is an N-methyl-D-aspartate (NMDA) receptor agonist, and thus considered to be potentially neurotoxic and contributing to the development of MDD (Muller and Schwarz, 2007). Conversely, KYNA is an NMDA receptor antagonist and is generally considered neuroprotective (Muller and Schwarz, 2007, Myint et al., 2007). IFN-α administration has been associated with an up-regulation of IDO, leading to reduced levels of serum tryptophan and serotonin (Bonaccorso et al., 2002a, Wichers et al., 2005). A recent genetic study has also shown that a polymorphism in the promoter region of the gene encoding IDO is associated with depressive symptoms during IFN-α therapy (Smith et al., 2011a). Interestingly, Capuron et al. reported that IFN-α-induced decreases in tryptophan, correlated with depressive, anxiety and cognitive symptoms but not the neurovegetative symptoms induced by IFN-α (Capuron et al., 2003a). This is similar to the results reported above for CRH and suggest that serotonin and CRH pathways may interact in mediating the development of mood and cognitive symptoms during IFN-α treatment (Capuron and Miller, 2004).

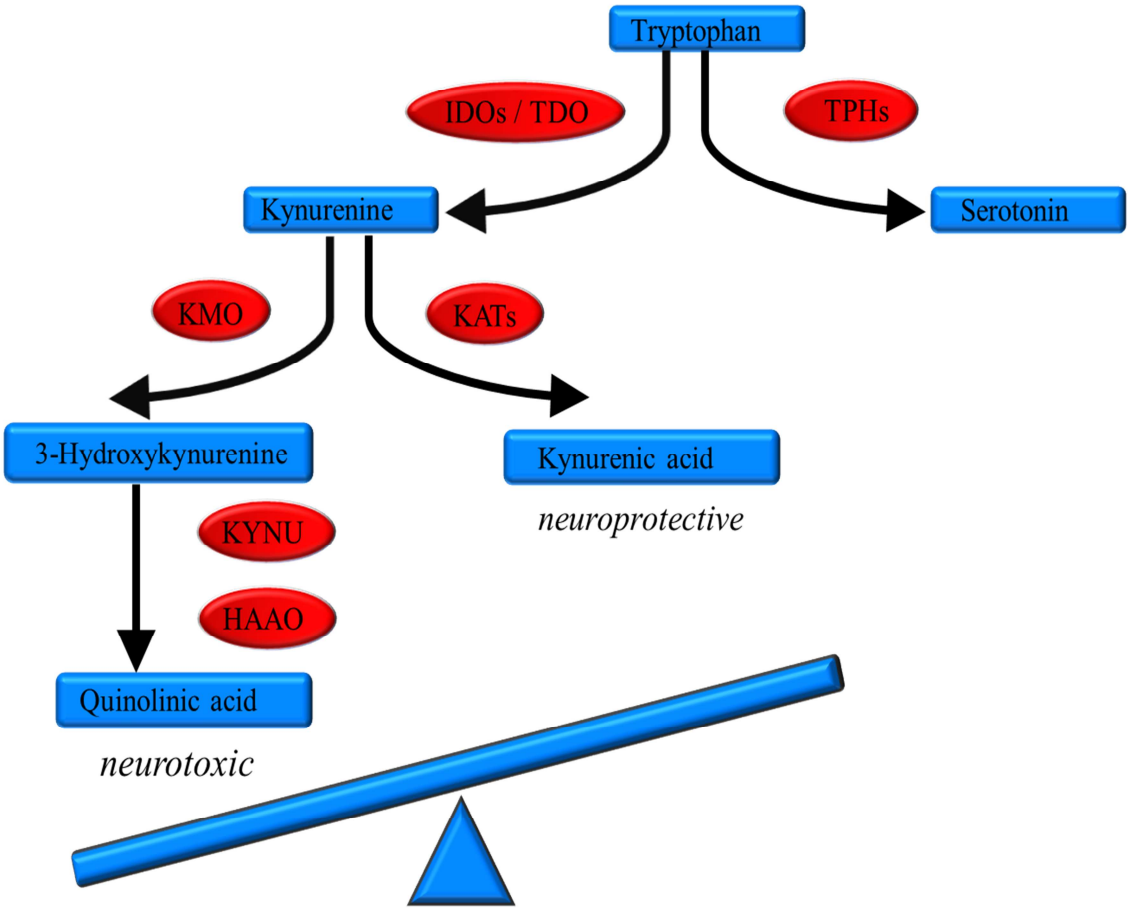


Figure 1.5 The metabolism of tryptophan

Tryptophan is synthesised into serotonin by tryptophan hydroxylase (TPH) or broken down in to kynurenine by the enzymes indoleamine 2,3- dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). Kynurenine is further metabolised by either kynurenine aminotransferases (KATs) resulting in kynurenic acid, or by kynurenine 3-monooxygenase (KMO) resulting in 3-hydroxykynurenine. 3-hydroxykynurenine is further metabolised by kynureninase (KYN) and subsequently synthesised into quinolinic acid (QUIN) via the activation of 3-hydroxyanthranilate 3,4-dioxygenase (HAAO). Figure adapted from (Zunszain et al., 2012b): Inflammation and Depression. *Current topics in behavioural neurosciences.* [Epub ahead of print].

As shown in Figure 1.6, as well as the reductions in tryptophan levels, it has also been demonstrated that upon IFN-α administration, there is an increase in KYN and QUIN which in turn correlate with depression scores (Raison et al., 2010b). Furthermore, CSF levels of the serotonin metabolite 5-hyroxyindoleacetic acid have also been shown to significantly predict development of depressive symptoms (Raison et al., 2010b). Both the reduced peripheral availability of tryptophan (putatively leading to reduced serotonin synthesis in the brain) and the production of neurotoxic tryptophan metabolites are considered essential steps in the pathophysiological processes leading to IFN-α-induced depression (Capuron and Miller, 2004). In my PhD, I will investigate the relationship between levels of kynurenine and tryptophan pathway metabolites and the development of IFN-α-induced depression, as well as monitor the change in levels over the treatment course.

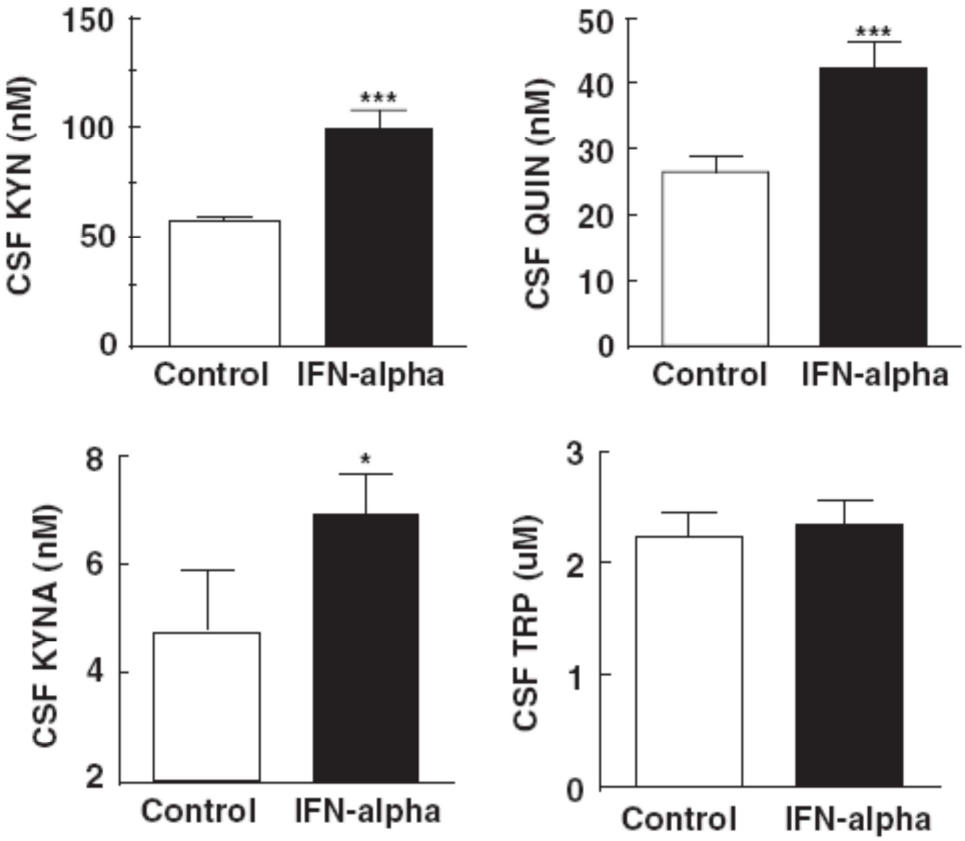


Figure 1.6 CSF levels of tryptophan and kynurenine pathway metabolites in HCV infected patients receiving IFN-α versus control patients

Cerebrospinal fluid (CSF) concentrations of kynurenine (KYN), quinolinic acid (QUIN) and kynurenic acid (KA) were significantly elevated in HCV infected subjects treated with IFN-α for 12 weeks when compared to untreated control patients. No differences were found between the groups in CSF concentrations of tryptophan (TRP). Figure from (Raison et al., 2010b): CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Molecular Psychiatry, 15,* 393-403.

1.3.8.3 Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) play an important role in MDD (Freeman et al., 2006) as well as in cytokine-induced sickness behaviour (Kozak et al., 1997). Specifically, societies with a high consumption of omega-3 PUFA rich foods such as fish, appear to have a lower prevalence of MDD (Tanskanen et al., 2001). Furthermore, there is evidence for lowered levels of omega-3 PUFAs including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the serum, fat tissues and brains of MDD patients (Lin et al., 2010, Maes et al., 1999). Studies have also shown evidence for a higher ratio of omega-6 PUFAs; such as arachidonic acid (AA), to omega-3 PUFAs to be associated with MDD (Frasure-Smith et al., 2004, Tiemeier et al., 2003).

Administration of omega-3 PUFAs have been shown to significantly improve depressive symptoms in MDD patients (Lesperance et al., 2011). A meta-analysis of the placebo controlled double-blind trials of omega-3 PUFAs in MDD showed that EPA has significant antidepressant activity although this is dependent on severity of depression (Lin et al., 2012, Lin and Su, 2007). One possible explanation for the association between depletions in omega-3 PUFAs and MDD, and the clinical efficacy of omega-3 PUFAs administration, is the fact that omega-3 and omega-6 PUFAs modulate immune functions (Leonard and Maes, 2012). Omega-3 PUFAs, attenuate prostaglandin E2 (PGE2) synthesis (See Figure 1.7) and the production of monocytic and T cell cytokines, including IL-1, IL-6, TNF-α and interferon-gamma (IFN-γ) (Maes et al., 1999, Su, 2009). The depletion of omega-3 PUFAs and the relatively higher omega-6 contents seen in MDD may cause an increase in the production of pro-inflammatory cytokines and T cell cytokines, and therefore take part in the immune

pathophysiology of MDD (Leonard and Maes, 2012). Another possible explanation is the regulation of neurotransmitters and signal transduction by PUFAs. DHA has been shown to be associated with increased neuronal membrane stability and functions of serotonin and dopamine transmission which could have implications for the development of MDD (Chalon, 2006). Omega-3 PUFAs can also have an effect on brain-derived neurotrophic factor (BDNF) which is known to be involved in synaptic plasticity as well as providing neuroprotection, and enhancing neurotransmission (Ikemoto et al., 2000) all of which are relevant for the development of MDD.

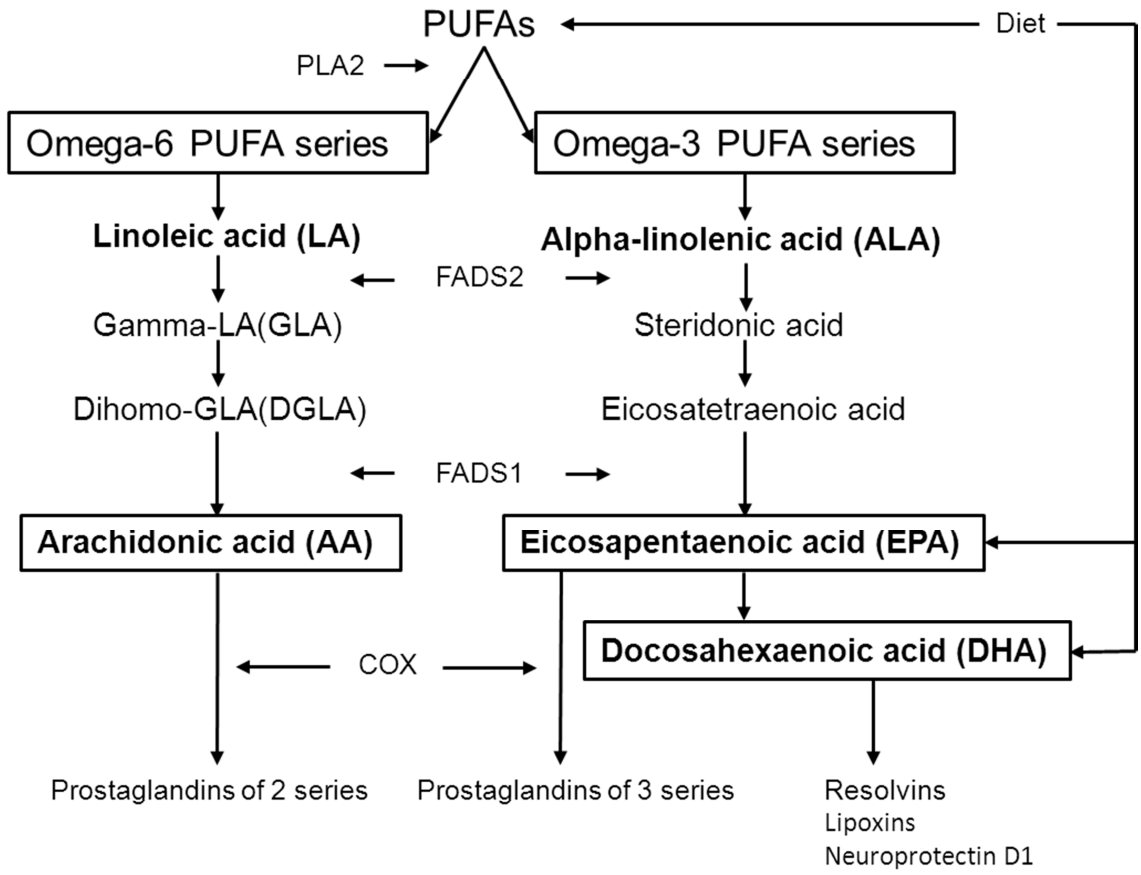


Figure 1.7 The metabolism of polyunsaturated fatty acids (PUFAs)

Figure adapted from (Su., 2009): Polyunsaturated fatty acids in interferon-induced depression. *PhD thesis.*

Only a few studies have investigated the role of PUFAs in the development of IFN-α-induced depression. As shown in Figure 1.8, a recent study found that lower baseline DHA levels and higher omega-6/omega-3 ratio predicted depression incidence (Lotrich et al., 2012). Furthermore, there is also evidence for the involvement of genetic polymorphisms in two key enzymes of PUFA metabolism and PGE2 synthesis; phospholipase A2 (PLA2) and cyclooxygenase-2 (COX2) (Su et al., 2010). Patients who developed IFN-α-induced depression had a higher frequency of the PLA2 BanI G/G or COX2 rs4648308 A/G genotypes. Interestingly, these “at risk” genotypes were also associated with lower levels of DHA and EPA, at baseline and during IFN-α treatment, suggesting that increased reactivity of inflammatory processes is fundamental in the development of depressive symptoms. In my PhD, I will investigate whether baseline levels of omega-3 and omega-6 fatty acids are associated with later development of IFN-α-induced depression. Moreover, I will also assess changes in omega-3 and omega-6 fatty acid levels during IFN-α therapy.

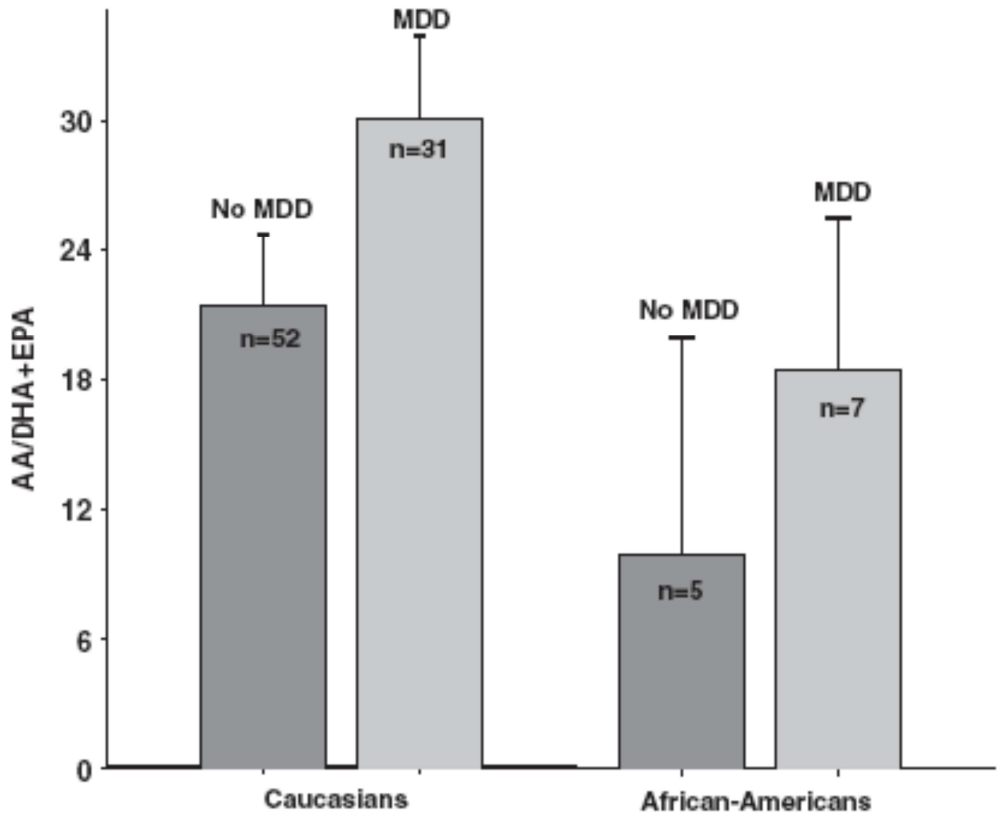


Figure 1.8 The ratio of AA/DHA+EPA at baseline in patients with and without IFN-α-induced depression

The ratio of the omega-6 fatty acid arachidonic acid (AA) to the combined levels of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) at baseline is associated with subsequent major depression (MDD) development during IFN-α treatment. Figure from (Lotrich et al., 2012): Elevated ratio of arachidonic acid to long-chain omega-3 fatty acids predicts depression development following interferon-alpha treatment: Relationship with interleukin-6. *Brain, Behaviour and Immunity, 31,* 48-53.

1.3.8.4 Gene expression

Finally, an emerging and useful method to investigate the pathogenesis of MDD is the use of peripheral blood to measure the expression levels of genes. This is a useful approach in biomarker identification, with opportunities for both hypothesis-driven biomarker search and for hypothesis-free “transcriptomics”-based discovery (Sunde, 2010). “Blood gene expression” usually refers to “intracellular RNA from blood”, and it is technically associated, in most cases, with two approaches: the use of tubes for blood collection that stabilize mRNA from all cells in the blood; and the extraction of mRNA from separate distinct blood cell populations. What is really of importance for researchers is whether blood mRNA can be used as a proxy for mRNA expression in other tissues that are more relevant to the pathogenic processes of interest – in psychiatry and neuroscience, the brain. In this regard, peripheral blood gene expression is very promising, as several studies have shown that blood cells share more than 80% of the transcriptome with other body tissues, including the brain (Liew et al., 2006). For example, Sullivan and colleagues compared the transcriptional profiling of 79 human tissues, including that of whole blood and of several brain areas. They, demonstrated that whole blood shares significant gene expression similarities with multiple brain tissues, in particular for genes encoding for neurotransmitter receptors and transporters, stress mediators, cytokines, hormones, and growth factors, all of which are relevant to MDD (Sullivan et al., 2006). As such, investigating peripheral blood gene expression appears to be a useful tool for assessing and understanding MDD.

Only a few studies have employed this technique in IFN-α-induced depression. In mRNA from peripheral blood mononuclear cells (PBMCs), levels of TNF-α

were found to remain stable between baseline and treatment week 4 in patients who developed IFN-α-induced depression, whereas in patients who did not develop depression levels were seen to decrease. Concomitantly, IL-10 levels decreased in depressed patients and increased in non-depressed patients (Krueger et al., 2011). Again using mRNA from PBMCs, depression as well as fatigue during IFN-α treatment were found to be associated with an increase in the expression of 2'-5'-oligoadenylate synthetase 2 (OAS2), a gene involved in the innate immune response to viral infection (Felger et al., 2012). Finally, a recent study analysing mRNA extracted from whole blood using PAXgene Blood RNA Tubes (PreAnalytiX, Switzerland), found that pre-treatment up-regulation of TNF receptor associated factor-6 (TRAF6) and down-regulation of transforming growth factor beta-1 (TGF-β1), predicted the development of depression during IFN-α treatment (Birerdinc et al., 2012). In my PhD, I will conduct microarray analysis in order to identify genes which are differentially expressed at baseline in patients who later develop IFN-α-induced depression when compared to those who do not. In this way, I will be able to identify potential predictors of IFN-α-induced depression using a hypothesis-free approach. Furthermore, I will also investigate differences in candidate genes, and monitor gene expression changes as a result of IFN-α treatment.

1.4 Current clinical practice and management

Understanding the underlying mechanisms of IFN-α-induced depression is essential in order to increase the accuracy with which vulnerable patients are identified. However, judgements of clinical experts are likely to remain indispensable in identifying vulnerable patients and providing care. New biomarker tools and methods will have to be integrated with current clinical practices, and so it is important to understand how clinical experts make decisions. Different theoretical perspectives have been used to study health care practitioners' decision making. Many approaches focus on the factors that determine how the most accurate decisions are made. However, emerging studies of how experts make decisions in naturalistic contexts has challenged these notions and provided evidence that decisions made outside the laboratory do not involve experts generating and weighting lists of action options. Researchers working in the naturalistic decision making (NDM) tradition have found that experts make decisions by recognising patterns and matching these to known courses of action (Hoffman and Millitello, 2009). Based on this, one stream of my PhD will assess the decision making processes that take place in the identification and monitoring of IFN-α-induced depression.

1.4.1 The Naturalistic Decision Making (NDM) framework

The focus of NDM studies of decision making is how experts make decisions in the real world. This perspective assumes that decisions in the real world involve ill-structured problems, uncertainty, time constraints, high stakes, multiple actors, organizational goals and action/feedback loops (Zsambok, 1997), and medical decision making shares many of these features. NDM approaches also emphasise the importance of the context in shaping decisions, and focus on the

information that is sought and attended to in the assessment of the situation (Hedberg and Satterlund Larsson, 2003). Using these insights, depression risk assessment in HCV patients can be viewed as a complex cognitive task which involves on-going decision making about patients' risk factors and their responses to treatment. Moreover, scientific knowledge about how to predict which patients will develop IFN-α-induced depression and which will not, is incomplete, and patients are not completely known to clinicians, so clinicians work with ill-defined and incomplete information. In my PhD, I will use the NDM approach as a framing perspective, to improve our understanding of staff experiences of, and attitudes towards the identification and monitoring of IFN-α-induced depression.

1.5 Aims and hypotheses of the study

1.5.1 Clinical and biological effects of IFN-α

IFN-α induces a broad range of neuropsychiatric side-effects (Raison et al., 2005b, Udina et al., 2012) and has effects on a variety of biological systems (Capuron and Miller, 2011, Raison et al., 2008). As such, a primary aim of this thesis is to monitor the impact of IFN-α treatment on a number of clinical and biological parameters. Specifically, I predict that:

- IFN-α will lead to an increase in depression, fatigue, stress and anxiety scores.
- IFN-α will lead to a decrease in health status and well-being measures.
- IFN-α will lead to a decrease in tryptophan levels, with a subsequent increase in kynurenine and its neurotoxic metabolites, and a decrease in the levels of the neuroprotective metabolite; kynurenic acid.
- IFN-α will lead to a decrease in the level of omega-3 PUFAs and an increase in omega-6 contents.
- IFN-α will lead to a number of gene expression changes particularly increased expression of genes involved in: tryptophan metabolism, PUFA metabolism and inflammation, and reduced expression of genes involved in GR functionality and neuroplasticity.

1.5.2 Clinical predictors of IFN-α-induced depression

Previous studies have found few clinical predictors of IFN-α-induced depression with the most widely investigated being a previous history of depression (Raison et al., 2005a, Smith et al., 2011b). However, known risk factors for MDD outside of the context of IFN-α treatment, such as a history of childhood trauma have

not yet been investigated in patients undergoing IFN-α therapy. As such, the second primary aim of this thesis is to identify novel clinical predictors of IFN-α-induced depression such as childhood trauma and illness perceptions. I will assess the contribution of a number of clinical and lifestyle factors on the subsequent development of IFN-α-induced depression. Specifically, I predict that:

- A previous history of depression, a family history of psychiatric illness and baseline psychopathology will be associated with the development of IFN-α-induced depression.
- Exposure to recent stressful life events as well as childhood trauma (physical and sexual abuse, parental loss or parental separation) will be associated with the development of IFN-α-induced depression.
- Negative illness perceptions will be associated with IFN-α-induced depression.

1.5.3 Biological predictors of IFN-α-induced depression

Several studies have shown IFN-α induces changes in the function of a number of biological systems including the immune and neuroendocrine systems, and these changes are related to depressive outcomes (Capuron et al., 2003b, Raison et al., 2009, Wichers et al., 2007). However, most of these have studied changes in these biological systems as a result of IFN-α, rather than assess baseline predictors. The third primary aim of this thesis is to investigate the baseline levels of a number of biological variables on the development of IFN-α-induced depression. I will investigate a number of biological systems and

furthermore, identify novel contributors through the use of microarray which offers a hypothesis-free approach. Specifically, I predict that:

- Increased cortisol awakening response as well as increased cortisol during the day will be associated with IFN-α-induced depression.
- Lower levels of tryptophan and higher kynurenine metabolite contents will be associated with the development of IFN-α-induced depression.
- Lower omega-3 PUFAs and higher omega-6 PUFAs will be associated with the development of IFN-α-induced depression.
- Increased expression of genes involved in tryptophan metabolism, PUFA metabolism and inflammation accompanied by reduced expression of genes involved in GR functionality and neuroplasticity will be associated with associated with IFN-α-induced depression.

1.5.4 Qualitative assessment of current clinical practice

Despite the potential to identify clinical and biological predictors of IFN-α-induced depression, the judgements and decisions of clinical experts will still be vital in identifying vulnerable patients and providing care, at least in the short term. As such, it is important to understand how clinical experts make decisions and how clinical teams co-ordinate their activities to care for patients. The final aim of this thesis is to gain an in-depth understanding of staff experiences of, and attitudes towards the identification and monitoring of IFN-α-induced-depression, and their decision-making processes. Specifically, I will investigate the following questions:

- What factors do clinical nurse specialists see as important in determining risk of developing depression, at initial consultation and in on-going monitoring?
- What actions do clinical nurse specialists take?
- What are the sources of uncertainty for clinical nurse specialists and available reduction strategies?

2 Methods

2.1 Study on patients undergoing IFN-α therapy

2.1.1 Study Design

A prospective cohort design was used to investigate the effects of IFN-α therapy. Patients were evaluated at baseline (week 0) and after 4, 8, 12, 16, 20 and 24 weeks of IFN-α treatment.

2.1.2 Participant Selection

Patients were recruited from the outpatient liver departments of three London hospitals: King's College Hospital, Guy's and St. Thomas' Hospital and St. George's Hospital. Eligible patients were adult patients with chronic hepatitis C virus (HCV) infection who were due to commence combination antiviral therapy with IFN-α and ribavirin. All patients received combination therapy for at least 24 weeks. This comprised of weekly subcutaneous IFN-α injections (1.5 μg per kg of body weight) and daily ribavirin tablets (800 to 1400 mg orally per day in 2 divided doses). Exclusion criteria included age below 18 years, any autoimmune disorder, any cause for liver disease other than HCV, current use of antidepressants, lack of English language and co-infection with HIV or hepatitis B. Written informed consent was obtained from all participants after a complete explanation of the study, a presentation of a participant information sheet and an opportunity to ask questions. The study was approved by the King's College Hospital Research Ethics Committee (Ref: 10/H0808/30). All patients were recruited from September 2010 to May 2012 with data from the last treatment week 24 assessment collected by November 2012. A total of 58 participants were recruited however, 10 participants only completed the baseline assessments as they either withdrew their participation in the study or

their treatment was stopped within the first 4 weeks due to extreme adverse effects. Their data were not included in this PhD; therefore, the final sample of this study comprises 48 patients (see Results).

2.1.3 Clinical data collection at baseline

Several assessments were conducted at the baseline, immediately before IFN-α therapy, in order to identify potential risk/predictive factors for the development of IFN-α-induced depression. Copies of all psychometric scales can be found in the Appendix.

2.1.3.1 Socio-demographic Data

Socio-demographic data (age; gender; self-rated ethnicity; level of education achieved; current relationship status and current employment status) were collected at baseline using a modified version of the MRC Socio-demographic Schedule (Mallett et al., 2002). As some variables were characterised by small cell sizes it was necessary to collapse categories. As such, I created dichotomised variables to consolidate multiple classes of data: for ethnicity, looking at White British individuals versus all other ethnicities; for level of education, looking at university degree versus all others; for current relationship status, looking at single versus all others and for current employment status, looking at part-time or full-time employment versus unemployed.

2.1.3.2 Mini International Neuropsychiatric Interview (MINI)

The MINI was administered at baseline in order assess patients for a current depressive episode or a previous history of MDD; it was also used at follow-up assessments for the detection of new onset cases of depression during IFN-α

therapy. The MINI is a structured diagnostic interview for psychiatric disorders according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) and the International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10). With an administration time of approximately 15 minutes, the MINI was designed to meet the need for a short yet accurate, structured psychiatric interview for use in multi-centre clinical trials and epidemiology studies, and to be used as a first step in outcome tracking in non-research clinical settings (Sheehan et al., 1998). The interview includes diagnoses of 19 disorders, including 17 Axis I disorders, a suicidality module and one Axis II disorder: antisocial personality disorder. For the purpose of this study, we only focused on the detection and diagnosis of major depressive episode and not any other psychiatric disorders. All researchers were trained in administering the MINI with the use of training videos. The training videos were comprised of actors simulating symptoms of depression that are consistent with the information assessed by the MINI.

2.1.3.3 Family History

Information regarding family history of psychiatric diagnoses was obtained using the Family Interview for Genetic Studies (Maxwell, 1992) and supplemented by medical notes where available.

2.1.3.4 Childhood Experiences of Care and Abuse (CECA)

A modified version of the Childhood Experiences of Care and Abuse (CECA) Questionnaire was used to collect information about childhood trauma. The CECA questionnaire is a self-report measure designed to elicit information concerning childhood experiences before the age of 17 (Bifulco et al., 2005)..

This includes information about parental loss, separation from parents for 6 months or more, physical and sexual abuse. Cut-off points were utilised to dichotomise responses on physical and sexual abuse variables. Using the cut-off points published by Bifulco et al., physical abuse was defined as repeated exposure to physical violence, from either the main mother or main father figure before the age of 17 years. In order to be considered ‘severe’, these incidents had to meet at least two of the following criteria; (a) being hit with a belt/stick or being punched or kicked; (b) resulting in an injury, including broken limbs, black eyes or bruising; (c) the perpetrator was considered to be out of control. Mild forms of punishment such as being smacked or hit with a slipper were excluded. Sexual abuse was defined as when at least one of the screening questions for sexual abuse was present (“When you were a child or teenager, did you ever have any unwanted sexual experiences?”, “Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17?”, “Can you think of any upsetting sexual experiences before age 17 with a related adult or someone in authority, e.g., a teacher?”). A composite variable was also created by adding together presence of one of the four dichotomised variables considered (loss of parents, separation from parents for 6 months or more, severe physical abuse and presence of sexual abuse); the score of this variable ranged from 0 (absence of any childhood trauma) to 4 (presence of all four types of childhood trauma investigated). This variable was then further dichotomised as 0 if no childhood trauma was experienced and 1 if one or more type of childhood trauma was experienced.

2.1.3.5 Brief Life Events (BLE)

The Brief Life Events questionnaire was administered to assess recent stressful events (Brugha and Cragg, 1990). This is a self-report questionnaire examining the incidence of 12 categories of negative life events over the previous 6 months. It assesses life stressors involving moderate or long-term threats such as illness or injury, the death of a close friend or relative, unemployment, financial loss and loss of important relationships. The questionnaire involves the reporting of any of the 12 events if they took place in the previous six months, together with a score of the emotional impact of each event at that time ('how bad was it at that time: "not too bad", "moderately bad" "very bad"'), allowing an assessment of both the number and the emotional impact of stressful life events. A dichotomised variable was created with 0 if no life events were experienced in the previous 6 months, and 1 if one or more type of life event was experienced in the previous 6 months prior to baseline.

2.1.3.6 Substance Use

All patients were asked about substance use using a modified version of the Cannabis Experience Questionnaire (Section 2 of CEQ) (Barkus et al., 2006). This questionnaire allowed detailed assessment of life time pattern of substance use looking at data such as age at first use and frequency of use of substances including cannabis, amphetamines and opioids. In my sample 36 out of 48 patients reported using at least one type of substance, at least once in their lifetime. As such, I specifically focused on the use of opioids as a more severe form of substance use, and also due to the high risk of HCV transmission associated with intravenous drug use. I created a dichotomised variable with 0 if no lifetime use of opioids and 1 for a current or a history of opioid use.

2.1.3.7 Illness Perceptions (IPQ)

The Illness Perceptions Questionnaire (IPQ) is comprised of five scales measuring the five components of illness representation specified in Leventhal's self-regulatory model of illness. The five scales assess *identity* (the symptoms the patient associates with the illness), *cause* (personal ideas about aetiology), *time line* (the perceived duration of the illness), *consequences* (expected effects and outcome), and *cure control* (beliefs about potential for cure and control of the illness) (Weinman et al., 1996). Higher scores indicate a strong emotional response, perception that the illness is chronic, that it is cyclic in pattern, that it has serious consequences, and that control or cure is possible. Due to the fact that HCV infection is largely asymptomatic, I did not use the identity domain of this questionnaire in my data analysis.

2.1.4 Clinical data collection at follow-up assessments

The following clinical measures were used to assess symptoms preceding the baseline and at every subsequent monthly assessment in order to not only identify baseline risk/predictive factors but also monitor symptom development. Copies of all psychometric scales can be found in the Appendix.

2.1.4.1 Inventory of Depressive Symptomatology (IDS)

The IDS is a 30-item questionnaire asking subjects to rate how they have felt over the past week in order to assess frequency and, duration or severity of a wide range of depressive symptoms (Rush et al., 1986). The scale assesses all 9 symptom domains needed to diagnose a DSM-IV major depressive episode and includes items to assess melancholic and atypical symptom features as well as commonly associated symptoms such as anxiety or pain. The IDS is

scaled to allow the detection of milder levels of depression and excludes uncommonly encountered symptoms such as depersonalisation (Lam et al., 2006). Items on the IDS are scored on a 0-3 scale; however, respondents answer either question 11 or 12 (decreased appetite or increased appetite) and either question 13 or 14 (weight loss or weight gain). As such, the total score range is 0-84 with higher scores indicating greater symptom severity. The authors have suggested the following severity indications: <12, normal; 13-23, mild; 24-36, moderate; 37-46, moderate-severe and >47, severe (Rush et al., 1986).

2.1.4.2 Hospital Anxiety and Depression Scale (HADS)

The HADS is a 14-item self-report questionnaire designed to screen for the presence and severity of depression and anxiety symptoms in medical patients over the past week (Zigmond and Snaith, 1983). This questionnaire is comprised of a 7-item depression sub-scale and a 7-item anxiety sub-scale, both of which omit somatic symptoms in order to reduce the likelihood of false positives (Lam et al., 2006). For the purpose of this study, I focused only on the anxiety sub-scale. Items on the HADS are scored on a 0-3 scale, and each sub-scale of 7 questions is summed to give a total score range of 0-21. Scores in the range of 0-7 are considered normal; 8-10, mild; 11-14, moderate; and 15-21, severe.

2.1.4.3 Chalder Fatigue Scale (CFQ)

The CFQ has been widely used to measure the severity of fatigue and consists of 11 questions measuring fatigue-related symptoms over the previous month. The CFQ contains 7 items which address physical fatigue and 4 items

addressing mental fatigue. Items are scored on a 0-3 scale, giving total scores ranging from 0-33 with higher scores indicating more fatigue (Chalder et al., 1993). Scores of ≤18 are considered to be within normal range for fatigue (White et al., 2013, White et al., 2007).

2.1.4.4 Perceived Stress Scale (PSS)

The 10-item Perceived Stress Scale measures the degree to which situations in one's life are appraised as stressful (Cohen and Williamson, 1988). In this study, the 10-item version was used, where each item asks the subject to rate how often they have perceived an event in their life over the last month (e.g. "In the last month how often have you felt difficulties piling up so high that you could not overcome them?"). Response options are assessed using a 5-point scale (0=never to 4=very often). Four items are worded in the opposite direction (e.g. "In the last month how often have you felt that things were going your way?") and are reverse-scored. The total score is computed by summing all 10 items, with a range of 0-40.

2.1.4.5 Medical Outcomes Study Short-Form 36 (SF-36)

The SF-36 is a self-report measure of general health and has been extensively used to assess health status in patients with mood and anxiety disorders (Ware et al., 1993). It assesses both physical and emotional well-being based on how an individual has functioned over the previous 4 weeks. The SF-36 assesses 8 primary dimensions: physical functioning, physical role limitation, bodily pain, social functioning, mental health, emotional role limitation, vitality (energy versus fatigue) and general health. The items are scored in a yes/no fashion,

and on 3, 5 and 6-point scales. The 8 sub-scales have score ranges of 0-100, where higher scores indicate better health status.

2.1.5 Laboratory methods

2.1.5.1 Salivary Cortisol

Saliva samples were collected to measure salivary cortisol, at baseline and at treatment week 24 (end of treatment). I used a salivette device (Sarstedt, Leicester, UK) in which saliva is absorbed. Subjects were instructed to collect saliva samples by placing the salivette on their tongue for 60 seconds. Samples were collected at 6 time points in a single day; immediately after awakening (0 minutes) and 15, 30 and 60 minutes after awakening, and again at 12pm and at 8pm. Subjects were instructed to wake up before 10 am, to take the first sample while still in bed, and then not to have breakfast or brush their teeth during the first hour of awakening, and then again in the 30 minutes before taking the samples at 12pm and 8pm. This is in order to avoid falsely high cortisol values due to plasma exudates from minor bleeding in the oral cavity, or from meal-stimulated rises in cortisol. During collection, subjects were instructed not to touch the samples with their hands. At each time point the subjects were also instructed to write on “information sheets” provided (see Appendix), the time of collection, if they had accidentally had anything to eat or drink before taking the sample, and if they had experienced any difficult or tense situations before taking the sample. Samples were kept in the refrigerator overnight and then collected at the visit appointment or sent back in the post in the morning.

On arrival to the laboratory, the samples were frozen at -20℃. After thawing, saliva samples were centrifuged at 3000 rev/min for 15 minutes at room

temperature, which resulted in a clear supernatant of low viscosity. Determination of cortisol levels was achieved using the High Sensitivity Salivary Cortisol ELISA KIT from Salimetrics, following the recommended procedure. Briefly, 25 µl of saliva and standards were assayed in duplicates, by incubation on a microtitre plate coated with monoclonal antibodies against cortisol. Cortisol linked to horseradish peroxidase was then added, to compete with cortisol in the standards and unknowns for the antibody binding sites. After incubation, unbound components were washed away and bound cortisol peroxidase measured by reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. The amount of cortisol peroxidase detected, as measured by the intensity of colour developed, is inversely proportional to the amount of cortisol present. Optical density was read at 450 nm with correction at 620 nm, using a Beckman Coulter DTX 880 plate reader, with Multimode Detection Software 2.0.0.12. Values of cortisol were calculated using SoftMax Pro 4.8 software, following a 4-parameter fit. All of the analyses were conducted by Dr. Patricia Zunszain, senior laboratory co-ordinator in my research group.

To investigate the cortisol response to awakening, I calculated the Area Under the Curve of the increase (AUCi) of cortisol levels after awakening, considering the changes of cortisol levels from baseline (0 minutes) to 15, 30, and 60 minutes after awakening (See Figure 2.1). To investigate the cortisol levels during the day, I calculated the Area Under the Curve (AUC) of cortisol levels at 0 minutes after the awakening, at noon and at 8pm (See Figure 2.2). Both formulas for the calculation of the AUCs were derived from the trapezoid formula (Pruessner et al., 2003).

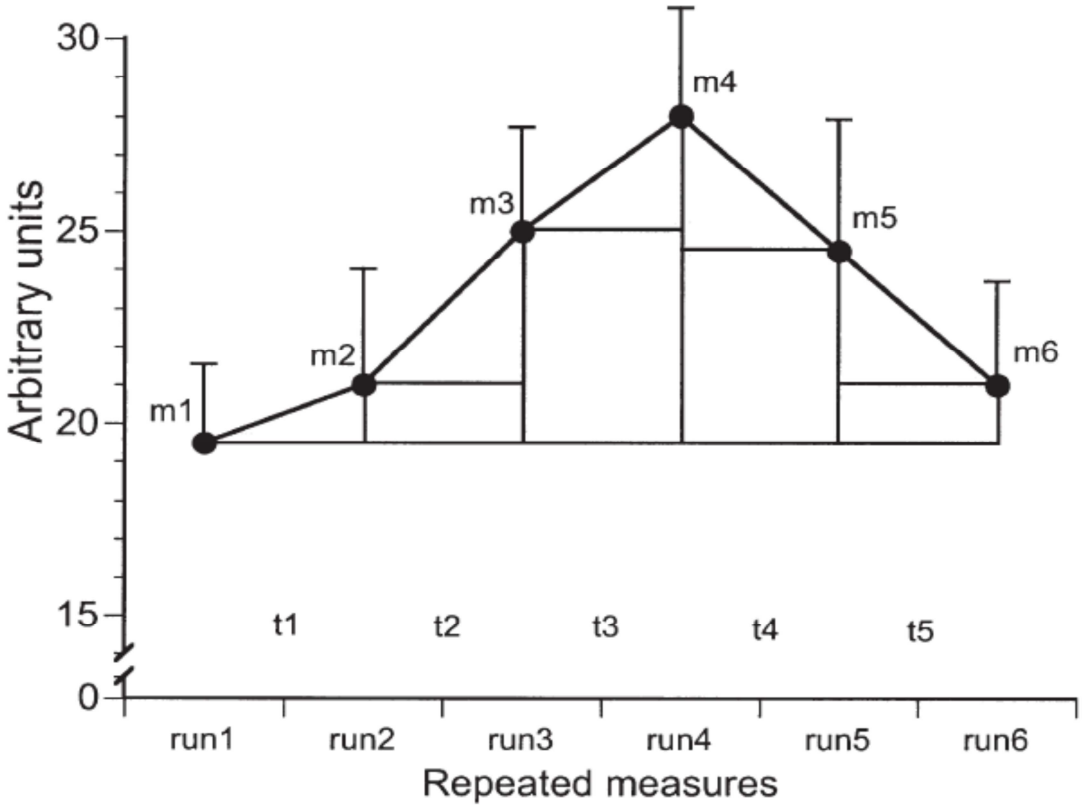


Figure 2.1 Area under the curve of the increase

The triangles and rectangles illustrate the composition of the area under the curve with respect to the increase (AUCi). *m*1 to *m*6 denote the measurements and *t*1 to *t*5 denote the time interval between the measurements. In this example the time interval between the measurements is identical; however individual time intervals were different in my sample (e.g. for the awakening response; 15 minutes between the baseline and 15 measurements, and the 15 and 30 measurements, but 30 minutes between the 30 and 60 measurements). Figure from (Preussner et al., 2003): Two formulas for the computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28,* 916-31.

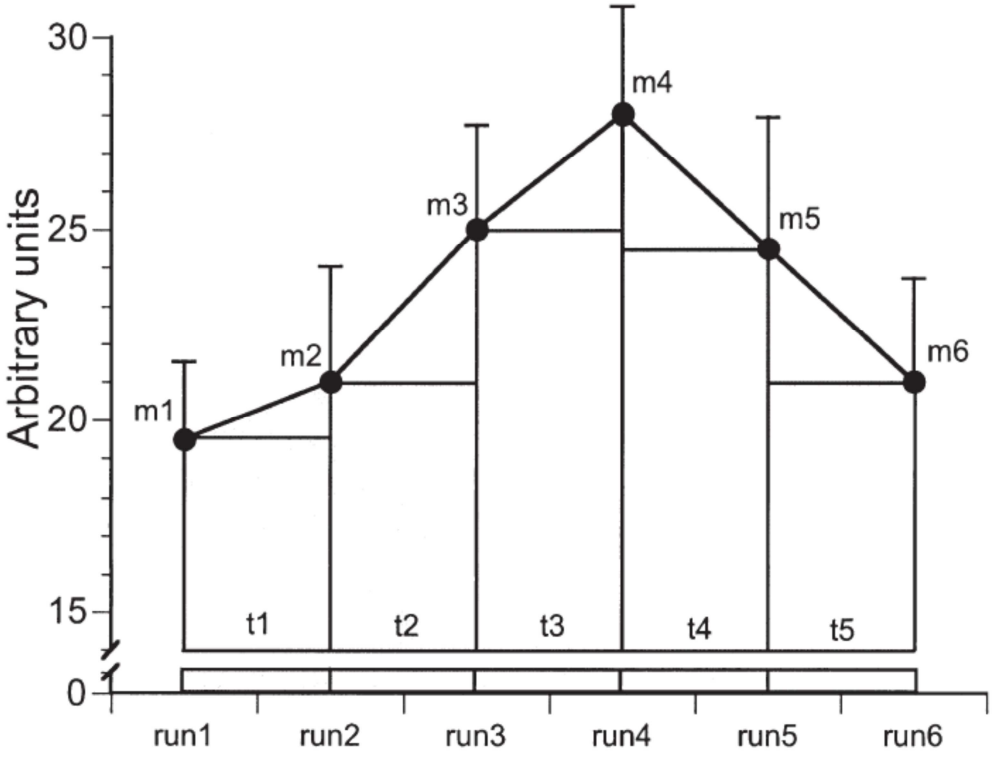


Figure 2.2 Area under the curve

The triangles and rectangles illustrate the composition of the area under the curve (AUC). *m*1 to *m*6 denote the measurements and *t*1 to *t*5 denote the time interval between the measurements. In this example the time interval between the measurements is identical; however individual time intervals were different in my sample (e.g. for the awakening response; 15 minutes between the baseline and 15 measurements, and the 15 and 30 measurements, but 30 minutes between the 30 and 60 measurements). Figure from (Preussner et al., 2003): Two formulas for the computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28,* 916-31.

2.1.5.2 Kynurenine and Tryptophan pathway

Blood samples were collected using 9ml VACUETTE® plasma separation, sodium heparin tubes, at baseline and at treatment weeks 8 and 24. On arrival to the laboratory, the samples were centrifuged at 500 rev/min for 10 minutes at room temperature, and then plasma removed and frozen at -80℃. After thawing, high performance liquid chromatography (HPLC) with a reverse phase c-18 column was used to measure plasma levels of tryptophan (TRP), kynurenine (KYN), kynurenic acid (KYNA) and 3-hydroxykynurenine (3-HK). The measurement was performed according to the method of Hervé et al. with some modifications (Herve et al., 1996). The recently published method using HPLC (Oades et al., 2010) was used to measure 3-HK. Briefly, KYN was detected spectrophotometrically at 365 nm. KYNA was detected fluorimetrically at an excitation wavelength of 334 nm and an emission wavelength of 388 nm. KYNA was analysed in plasma that was de-proteinised using perchloric acid. 3-HK was measured at a wavelength of 365 nm by UV detection. The 3-HK analysis method has been validated showing an absolute recovery of 85.8%, intra-day precision of 3.9%, and inter-day precision of 7.5%. The intra and inter-assay coefficients of variation ranged from 5% to 7% for all of the metabolites. All of the analyses were conducted by Dr. Aye-Mu Myint at Ludwig-Maximilians-University, Munich, Germany.

2.1.5.3 Gas Liquid Chromatography for PUFA analysis

Blood samples were collected using 2ml K3EDTA tubes at baseline and at all subsequent treatment weeks. On arrival to the laboratory, the samples were centrifuged at 1500 rev/min for 15 minutes at room temperature and then plasma removed and frozen at -80℃. After thawing, 100 µl of plasma was

aliquoted precisely in to glass tubes with Teflon-lined caps. An internal standard consisting of 50 µg to 300 µg of pentadechanoic acid dissolved in 2 ml of methanol-toluene (4:1) was mixed precisely with acetyl chloride at a ratio of 10:1. 220 µl of this mixture was added to the plasma samples and then subjected to methanolysis at 60℃ for 2 hours. After tubes had been cooled at room temperature, 5 ml of 6% potassium carbonate solution was slowly added to stop the reaction and neutralize the mixture. The tubes were then shaken and centrifuged, and an aliquot of the benzene upper phase was collected and placed in injection vials for analysis. Fatty acid methyl esters were analysed on a 25 mm x 22 mm internal diameter silica column (BP70X; SGE, Melbourne, VIC, Australia) using hydrogen as a carrier gas on an Agilent chromatograph 6890 (Agilent, Stockport, Cheshire, UK) in split mode (50:1). Initially, oven temperature was set at 160℃ for 4 minutes and then the temperature was increased by 10℃/min until 200℃ and held at this final temperature for 10 minutes. Peaks were integrated using ChemStation (revision B4.01) software. Fatty acids were identified by comparison with reference standards 189-1, 189-2 (Sigma, Poole, Dorset, UK) and by a methyl ester preparation of MAXEPA to identify long chain omega-3 fatty acids. The levels of PUFAs were generated as percentage of total fatty acids. I conducted all the analyses of PUFA levels.

2.1.5.4 Gene Expression

Blood samples were collected in PAXgene Blood RNA Tubes (PreAnalytiX, Switzerland) using standard protocols at baseline and at treatment week 4. After blood samples were drawn, PAXgene tubes were inverted 5-10 times and then kept upright at room temperature for 2 hours. After this, samples were placed in -20℃ for 48 hours and then transferred to -80℃ for storage until they

were processed. Isolation of total RNA (mRNA and miRNA) was performed using the PAXgene blood miRNA kit according to the manufacturer's recommended protocol (PreAnalytiX, Switzerland). In brief, blood was pelleted, washed, and then re-suspended with a lysis buffer and proteinase K to digest cellular proteins. Samples were then passed through PAXgene Shredder spin columns to homogenise the lysate and filter out cell debris. The supernatant of the flow-through was then passed through the PAXgene RNA spin column where the silica membrane selectively binds to the RNA. The RNA was DNase treated and after several wash steps it was eluted and heat-denatured. RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrophotometer (NanoDrop Technologies, USA).

Gene expression microarray assays were performed using Affymetrix® Human Gene 1.1 ST Array strips, on the GeneAtlas® platform according to the protocol (http://media.affymetrix.com/support/downloads/manuals/geneatlas_wt_expkit_manual.pdf). Partek Genomics Suite 6.6 software was used for data visualization, statistical testing of affymetrix CEL files and quality control. Data quality was assessed using histograms of signal intensities, scatter plots, and hierarchical clustering of samples. All samples passed the criteria for hybridization controls, labelling controls and 30/50 Metrics. Robust MultiChip Average method was used for background correction and Quantiles normalization. Summarization was performed using a median polish algorithm (Tukey, 1977).

Gene expression data were analysed using two approaches. Firstly, I used a hypothesis-free approach including pathway analysis using Ariadne Pathway Studio Software. Secondly, I used a candidate gene approach to investigate genes expression differences and changes based on evidence from previous studies. I selected genes known to be involved in tryptophan and PUFA metabolism (See Figure 1.5 and Figure 1.7), some of which have previously been implicated in the development of IFN-α-induced depression (Smith et al., 2011a, Su et al., 2010). I selected genes involved in inflammation, namely cytokines, for which altered serum levels have been demonstrated in IFN-α treated patients or in MDD outside of the context of IFN-α. Specifically, interleukin (IL) – 1, IL-2, IL-6 and their receptors, as well as IL-8, IL-10, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), have been previously demonstrated to be altered in the serum of IFN-α patients (Bonaccorso et al., 2001, Grungreiff et al., 1999, Loftis et al., 2008, Wichers et al., 2007, Wichers et al., 2006) or in gene expression studies of MDD patients (Cattaneo et al., 2012, Tsao et al., 2006). Interleukin 18 (IL-18) is an important modulator of immune responses that has been found to be elevated in MDD patients (Kokai et al., 2002, Merendino et al., 2002). Polymorphisms in the IL-28β gene have been shown to predict IFN-α treatment response (Ge et al., 2009) and one study has investigated the association between IL-28β and IFN-α-induced side-effects (Lotrich et al., 2011). As such, I also included IL-28β in my list of candidate genes. A previous gene expression study in IFN-α treated patients, found alterations in the expression of TNF receptor associated factor-6 (TRAF6) and transforming growth factor beta-1 (TGF-β1), and so these were also included in my list of inflammation related candidate genes (Birerdinc et al., 2012). Finally, I also selected genes which are involved in neuroplasticity.

Lower levels of brain-derived neurotrophic factor (BDNF) have been previously shown to be involved in IFN-α-induced depression (Kenis et al., 2010). Furthermore, gene expression levels of BDNF as well as other neurotrophic factors such as VGF and vascular endothelial growth factor (VEGF) have been shown to be altered in MDD patients when compared to controls (Cattaneo et al., 2012, Cattaneo et al., 2010, Iga et al., 2007, Pandey et al., 2010). The expression of the glucocorticoid receptor (NR3C1) and its chaperones and co-chaperones, such as FK506 binding protein (FKBP)-4 and FKBP-5 have also been shown to be altered in MDD patients when compared to controls (Cattaneo et al., 2012).

I completed all the RNA extraction, while the gene expression microarray assays and subsequent bioinformatics analyses were conducted by Dr. Annamaria Cattaneo, a post-doctoral researcher in the team expert in this research approach.

2.1.6 Data Analysis

All data were analysed using IBM SPSS statistical software version 20 and STATA version 10. Whenever appropriate, data are presented as Mean±SEM (standard error of the means). Graphs are presented as mean and 95% confidence intervals. The independent-samples *t* test was used to compare means of continuous variables between patients with and without IFN-α-induced depression. All comparisons of categorical data were examined using the chi-squared (x^2) test. Correlations between baseline values of clinical and biological data were tested with Pearson's analysis. ANOVA analyses were performed to

identify significantly different genes between patients with and without IFN-α-induced depression, and between baseline and treatment week 4.

Random intercept regression models with maximum likelihood effects were used to investigate changes in psychopathological scales and biological variables as a function over time. This method of analysis is an ideal way to model repeated measures as it can handle missing data. The dataset is changed from wide to long format and clustered by individual. Random intercept regression models with maximum likelihood effects were also used to assess the predictive effects of baseline demographic, psychosocial stress, cognitive, psychopathology, health status and biological factors on subsequent psychopathological scale scores. In order to give a clearer index of the variance explained by individual predictors, an overall R^2 value is given for significant predictors. All graphs are presented as means and 95% confidence intervals. Throughout the thesis statistically significant *P* values are indicated in **bold**.

It is important to note that due to the large number of predictor variables I have assessed, there is a potential for increased type 1 error as multiple comparisons were not corrected for. However, frequently used procedures such as the Bonferroni method are conservative, not appropriate for a large number of tests and diminish statistical power (Bland and Altman, 1995). Furthermore, methods to adjust for multiple testing in studies such as this which collect repeated measurements are rare as these comparisons occur for between-subject factors, within-subject factors, or both (Bender and Lange, 2001).

2.2 Qualitative study on nursing staff

2.2.1 Study Design

A qualitative interview study was conducted among clinical staff involved in the care of patients with hepatitis C receiving IFN-α therapy, using the perspective of naturalistic decision making.

2.2.2 Participants Selection

Purposive sampling was used to select participants most able to provide information relevant to the study objectives. The participants were staff members working in outpatient liver clinics in three large teaching hospitals in South London. All clinical nurse specialists (n=9) from all three centres were approached and agreed to participate. All were involved directly in the care of patients receiving IFN-α treatment and had at least one year's experience (mean 6.4 years, range 1-11 years) working in this field. Participants were initially approached via email. All participants gave written informed consent after receiving an information sheet detailing the nature and purpose of the research and being given an opportunity to ask questions.

2.2.3 Data Collection

A semi-structured interview guide was developed (See Appendix), and interviews lasting approximately 30 minutes were conducted individually with each member of staff. The interviews were organised into the following sections: background information on training and experience providing HCV care; current clinical practices/protocols for monitoring and identifying psychiatric side-effects, including referrals to psychiatrists; and personal attitudes to the use of decision making tools and access to resources. Standard

probes, such as verification, were used to fully clarify specific responses. All interviews were conducted jointly by myself and one other experienced social science interviewer; Dr. Naonori Kodate. All interviews took place between November 2010 and April 2011. Interviews were tape recorded, transcribed verbatim and then imported into the qualitative data analysis package (QSR NVivo 8.0 data analysis software) to facilitate data handling.

2.2.4 Data Analysis

Data were analysed using thematic framework analysis to identify key themes which were then used to code the interview data (Ritchie and Spencer, 1994). Framework analysis is a method that is particularly suited to studies in which at least some of the themes and concepts can be identified a priori (Dixon-Woods, 2011, Ritchie et al., 2003). Questions of relevance to the research aims are used as an initial thematic framework for the analysis, but the approach also allows for the inductive identification of emergent themes (Dixon-Woods, 2011, Pope et al., 2000). The initial coding framework was based on the interview schedule and further themes were identified as the analysis progressed. The framework was refined iteratively by comparing and discussing interpretations of the data and a final coding framework developed along with detailed descriptions of each theme to increase the reliability of coding. In order to further ensure the reliability of the analysis, Dr. Naonori Kodate and I independently examined portions of the transcripts and resolved any differences. The final coding framework consisted of major factors influencing identification and monitoring of depression and subsequent decision making.

2.2.5 My contribution

I contributed to the study design, protocol and set-up. This included completing the ethical approval, as well as R&D approvals from all three study sites. I collected approximately 80% of the data (clinical and biological), with help from placement students whom I supervised and co-ordinated for the remainder of the data collection. All of the cortisol samples were analysed by Dr. Patricia Zunszain, senior laboratory co-ordinator in my research group. I collected and processed approximately 80% of the blood samples, again with the help of placement students, in order to obtain plasma for kynurenine and tryptophan pathway analysis, as well as PUFA analysis. All of the kynurenine and tryptophan pathway metabolites were measured by Dr. Aye-Mu Myint at Ludwig-Maximilians-University, Munich, Germany. I conducted all the analyses of PUFA levels. I also completed all of the mRNA extraction, before the microarray assays and subsequent bioinformatics analyses were conducted by Dr. Annamaria Cattaneo. For the qualitative study, I jointly conducted all the interviews with Dr. Naonori Kodate. I alone transcribed all of the interviews before jointly analysing the data. I alone completed all of the data entry as well as conducting the statistical analyses contributing to this thesis.

3 Results

In this Results section, I will first discuss the characteristics of the sample (socio-demographics, exposure to psychosocial stressors, illness perceptions, baseline psychopathology and baseline health status). Then I will look at the changes over time due to IFN-α treatment in the whole sample, for the clinical (depression, fatigue, stress, anxiety and general health status) and biological variables (cortisol, kynurenine and tryptophan pathway, PUFA levels and gene expression). Then I will look at the incidence of IFN-α-induced depression and compare the same clinical and biological variables, in patients with and without IFN-α-induced depression, at baseline and over the course of IFN-α treatment. This will be followed by investigating the above-listed clinical and biological variables as predictors of depression, fatigue, stress and anxiety during IFN-α treatment. Finally, I will present the results of the qualitative study looking at the predictors or risk factors for depression currently assessed by clinical nurse specialists, followed by methods of co-ordinating actions, sources of uncertainty and suggested areas of improvement.

3.1 Study on patients undergoing IFN-α therapy

3.1.1 Characteristics of the sample

Forty-eight patients were consented and completed at least one follow-up assessment during IFN-α treatment.

3.1.1.1 Socio-demographic characteristics

The sample had an age range of 18-63 with a mean age of 43.3. The sample was predominantly male (75%) and of a white British background (48%). At baseline, no patients met criteria for a current MDD diagnosis, were taking any antidepressant medication or were being treated for an anxiety disorder. However, 17 patients (35%) had a self-reported history of MDD and 13 patients (27%) had a family history of psychiatric illness. Furthermore, 17 patients (35%) had a history of substance use, specifically use of opioids. There was some overlap in these characteristics; 5 patients (39%) with a family history of MDD also had a self-reported history of MDD, and 7 patients (41%) with a history of substance use also had a self-reported history of MDD. Only 2 patients reported a history of all three of these characteristics. The sample was predominantly comprised of patients with HCV genotype 3 (65%). The mean baseline viral load of the sample (that is, the number of viral particles per ml of blood presented in millions) was 2.1±0.4. The severity of the liver disease was also measured by a fibroscan to assess scarring of the liver, which is rated as a specific score in kilopascals (KPa). In viral hepatitis, a score of less than 7 means no or insignificant liver fibrosis; a score of more than 12.5 KPa is severe fibrosis or cirrhosis, and intermediate results suggest moderate fibrosis. The sample had a mean fibroscan score of 8.9±1.2 indicating moderate fibrosis. These data are presented in Table 3.1.

Table 3.1 Socio-demographic characteristics

	Patients *n* = 48
Age (years)	
Mean±SEM	43.3±1.6
Gender	
Males	36 (75%)
Ethnicity	
White British	23 (48%)
Education Level	
University Degree	15 (31%)
Employment	
Unemployed	17 (35%)
Relationship Status	
Single	24 (50%)
History of MDD	17 (35%)
Family History	13 (27%)
Substance Use	17 (35%)
HCV Genotype	
1	11 (23%)
2	5 (10%)
3	31 (65%)
4	1 (2%)
HCV Viral Load (million)	
Mean±SEM	2.1±0.4
Fibroscan Score (kpa)	
Mean±SEM	8.9±1.2

3.1.1.2 Psychosocial stress characteristics

The psychosocial stress characteristics of the sample including recent life events within the previous 6 months, as well as childhood traumatic events are shown in Table 3.2. Almost half of the sample (46%) reported experiencing at least one stressful life event in the 6 months before initiation of IFN-α treatment. Nineteen patients (40%) reported experiencing at least one form of childhood traumatic event. The most frequently reported form of childhood trauma was parental separation as reported by 12 patients (25%).

3.1.1.3 Illness perceptions characteristics

Patients showed good illness coherence, with a mean score of 18.3 out of a possible 20. They also held strong beliefs that treatment is an effective way of controlling their illness, with a mean score of 21.4 out of a possible 25, as well as strong beliefs about their personal ability to control symptoms, with a mean score of 22.9 out of a possible 30. Patients' beliefs about the seriousness of their illness and its consequences were not as strong, with a mean score of 18.2 out of a possible 30, as were their emotional representations, with a mean score of 17.5 out of a possible 30. Participants perceived their illness to be acute rather than chronic, with a mean score of 14.7 out a possible 30, and also did not see their illness as being cyclical in nature, with a mean score of 9.2 out of a possible 20 for this dimension. These data are presented in Table 3.3.

Table 3.2 Psychosocial stress characteristics

	Patients ***n*** **= 48**
Brief Life Events	
Yes	22 (46%)
Parental Separation	
Yes	12 (25%)
Parental Loss	
Yes	5 (10%)
Childhood Physical Abuse	
Yes	7 (15%)
Childhood Sexual Abuse	
Yes	8 (17%)
Any Childhood Trauma	
Yes	19 (40%)

Table 3.3 Illness perceptions scores

	Patients ***n*** **= 48**
Timeline *(acute/chronic)*	14.7±0.9
Consequences	18.2±0.9
Timeline *(cyclical)*	9.2±0.5
Personal Control	22.9±0.5
Treatment Control	21.4±0.7
Illness Coherence	18.3±0.6
Emotional Representations	17.5±0.9

3.1.2 Psychopathological changes during IFN-α treatment

As part of the first aim of this thesis, I prospectively monitored the impact of IFN-α treatment on a number of psychopathological factors. Changes in depression, fatigue, stress and anxiety scores during IFN-α treatment are shown in Figure 3.1-Figure 3.4. Both depression and fatigue scores increased rapidly between baseline and treatment week 4 and remained elevated until the end of treatment. A random intercept regression model with maximum likelihood effects showed a significant effect of treatment week on increasing depression scores (Coefficient=0.1, p=0.041). Similarly, there was also a significant effect of treatment week on increasing fatigue scores (Coefficient=0.1, p=0.010).

Stress scores gradually increased between baseline and treatment week 12 and remained elevated until the end of treatment. There was a significant effect of treatment week on increasing stress scores (Coefficient=0.1, p=0.014). Finally, changes in anxiety scores were less pronounced but nonetheless there was a continuous gradual increase in scores between baseline and the end of treatment. There was a significant effect of treatment week on increasing anxiety scores (Coefficient=0.1, p=0.026).

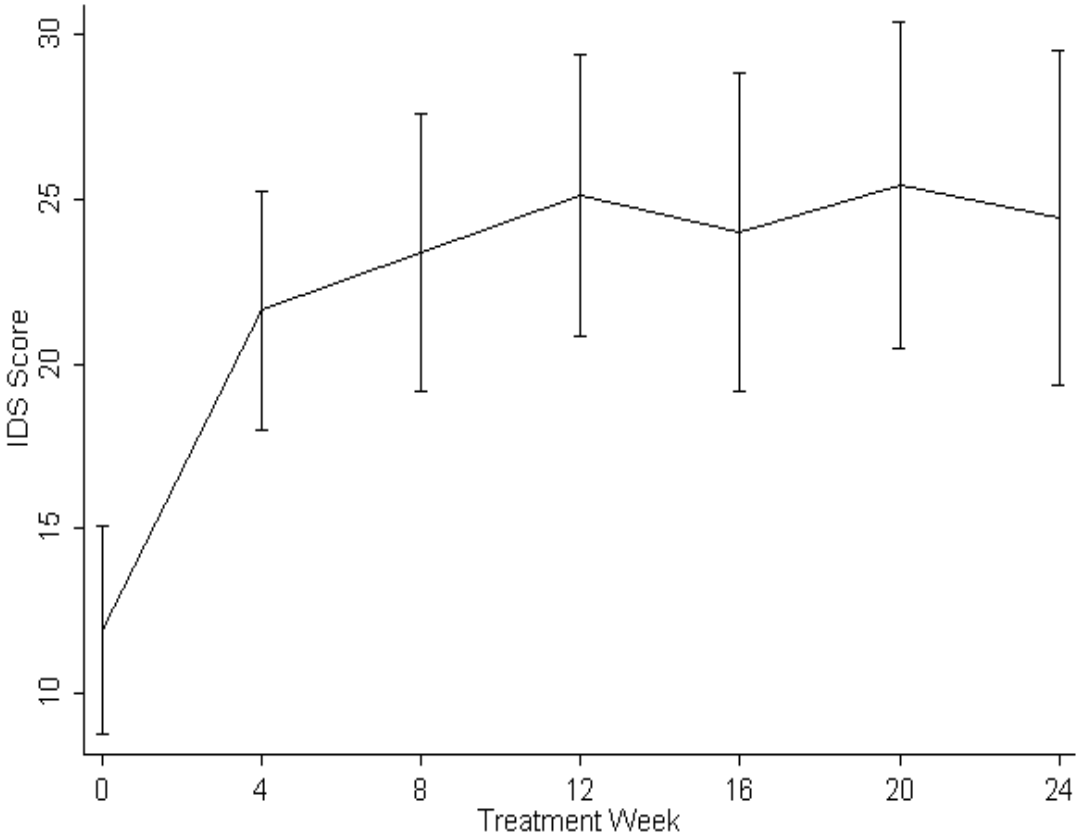


Figure 3.1 Changes in mean depression scores during IFN-α treatment

Changes in mean scores on the Inventory of Depressive Symptomatology (IDS) across the 24 weeks of treatment (*n* ranging from 37-48).

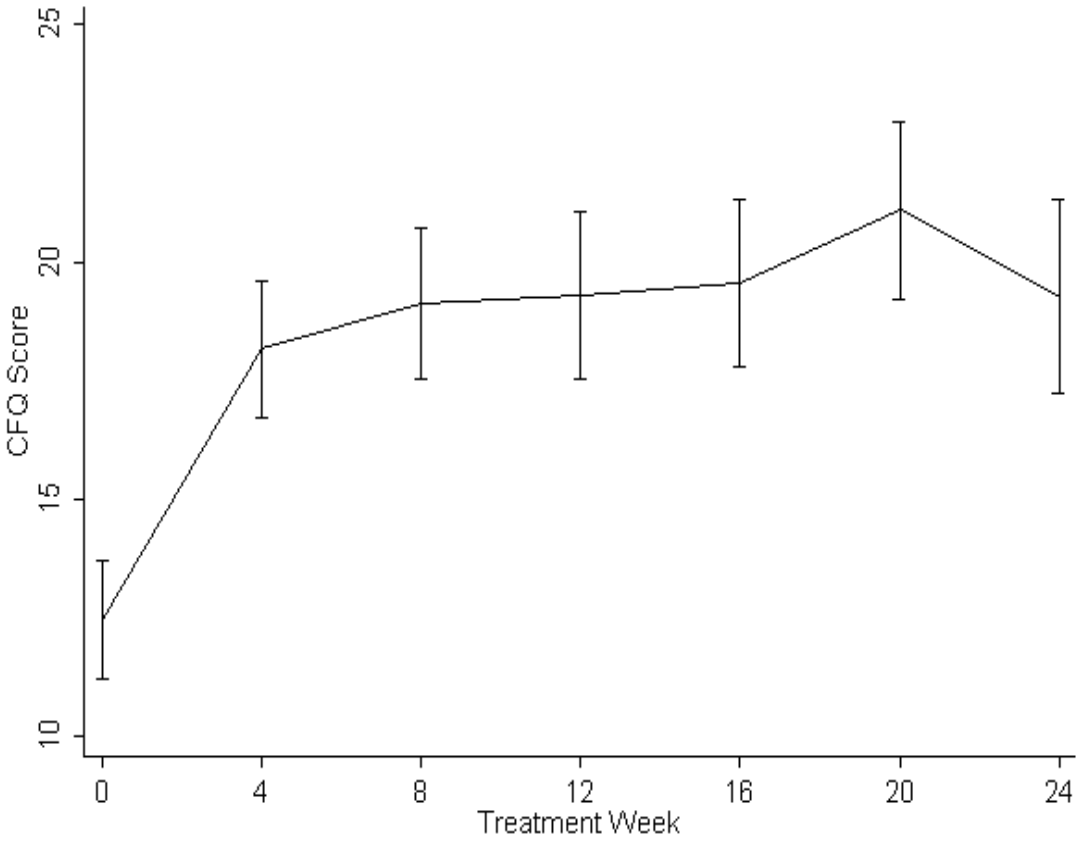


Figure 3.2 Changes in mean fatigue scores during IFN-α treatment

Changes in mean scores on the Chalder Fatigue Questionnaire (CFQ) across the 24 weeks of treatment (*n* ranging from 37-48).

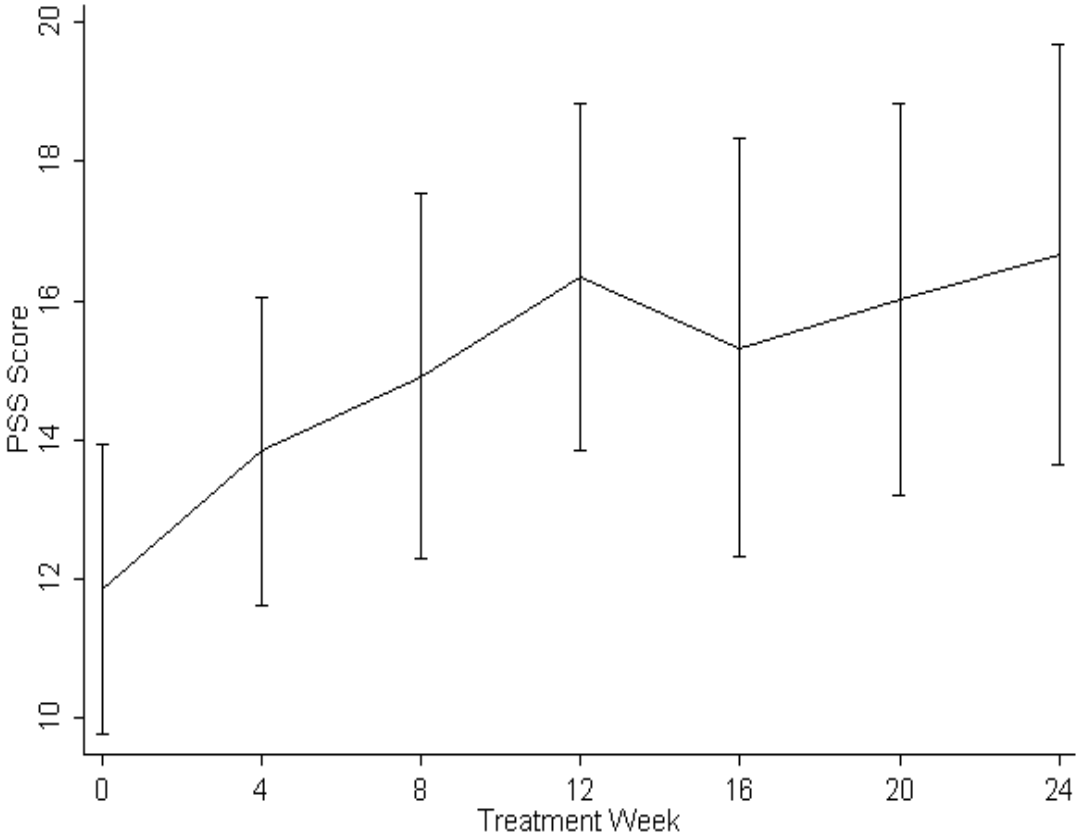


Figure 3.3 Changes in mean stress scores during IFN-α treatment

Changes in mean scores on the Perceived Stress Scale (PSS) across the 24 weeks of treatment (*n* ranging from 37-48).

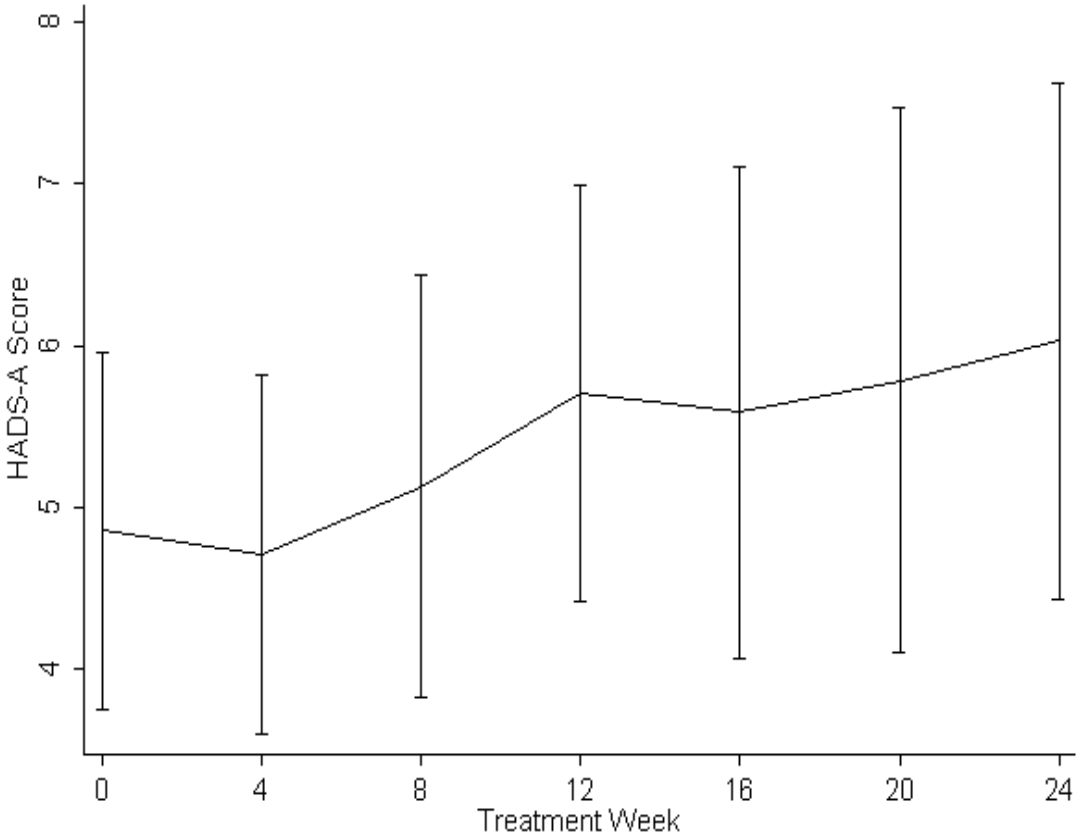


Figure 3.4 Changes in mean anxiety scores during IFN-α treatment

Changes in mean scores on the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) across the 24 weeks of treatment (*n* ranging from 37-48).

3.1.2.1 Changes in health status during IFN-α treatment

I also investigated the impact of IFN-α treatment on patients' health status. Changes in mean scores for the 8 health status dimensions of the SF-36 during IFN-α treatment are shown in Figure 3.5-Figure 3.12. As mentioned previously, for all 8 dimensions, higher scores represent better functioning with a score of 100 indicating optimal functioning/well-being. Over the course of IFN-α treatment, scores decreased across all 8 dimensions. There was a significant negative effect of treatment week on decreasing scores on the physical functioning, vitality, mental health and social functioning dimensions (Coefficient=-0.4, $p<0.001$; Coefficient=-0.3, $p=0.018$; Coefficient=-0.4, $p=0.001$ and Coefficient=-0.6, $p<0.001$, respectively). There were no significant effects of treatment week for scores on the physical role limitation, emotional role limitation, bodily pain or general health dimensions ($p=0.1$, $p=0.1$, $p=0.1$ and $p=0.1$, respectively).

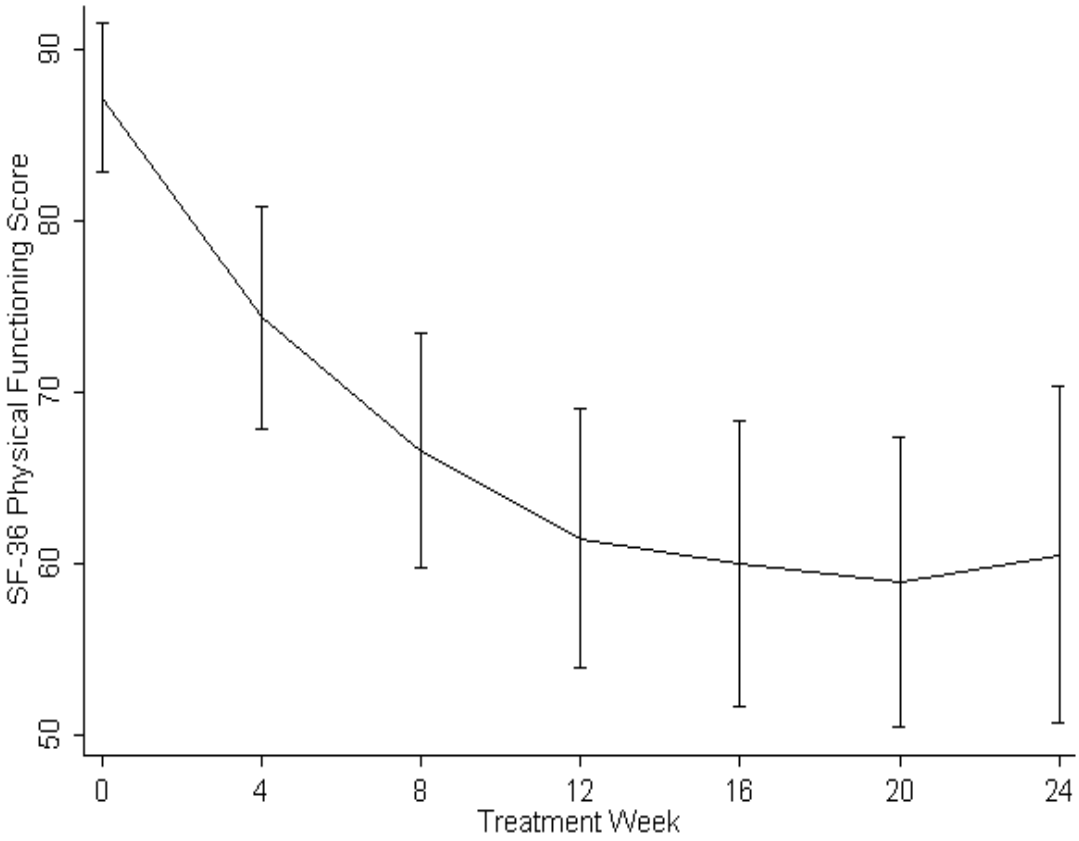


Figure 3.5 Changes in mean physical functioning scores during IFN-α treatment

Changes in mean scores on the physical functioning dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

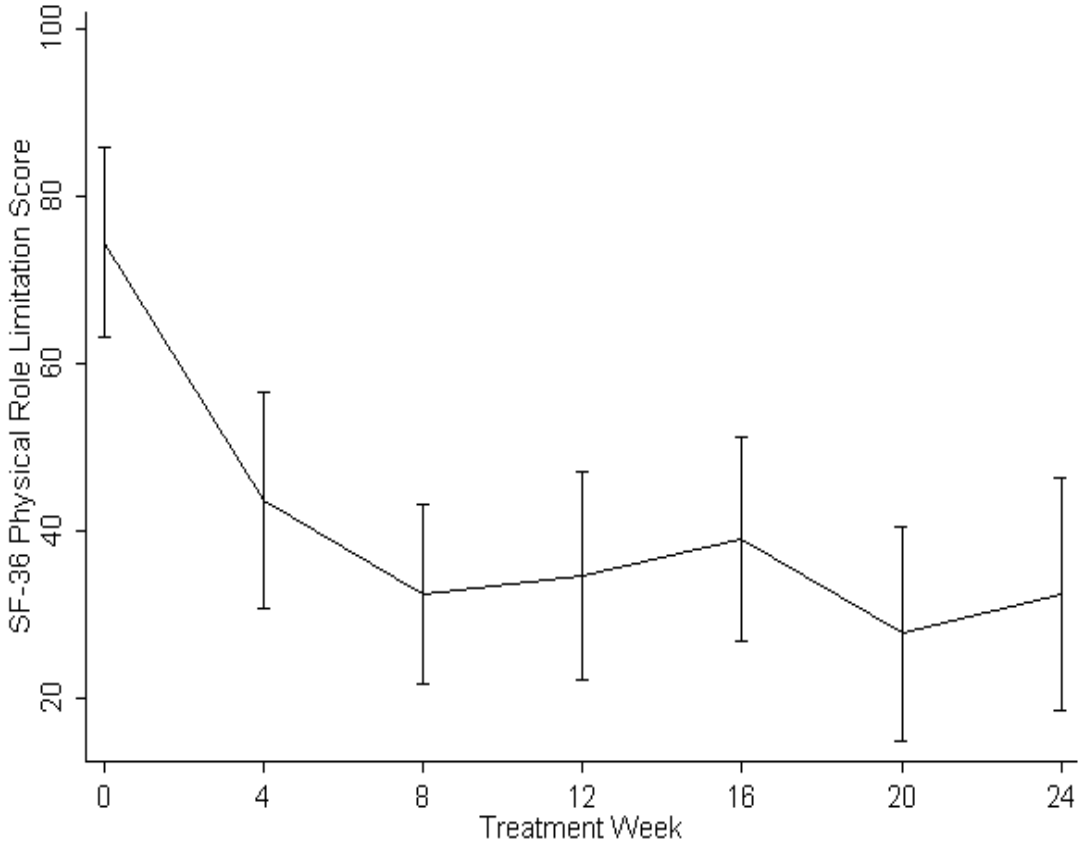


Figure 3.6 Changes in mean physical role limitation scores during IFN-α treatment

Changes in mean scores on the physical role limitation dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

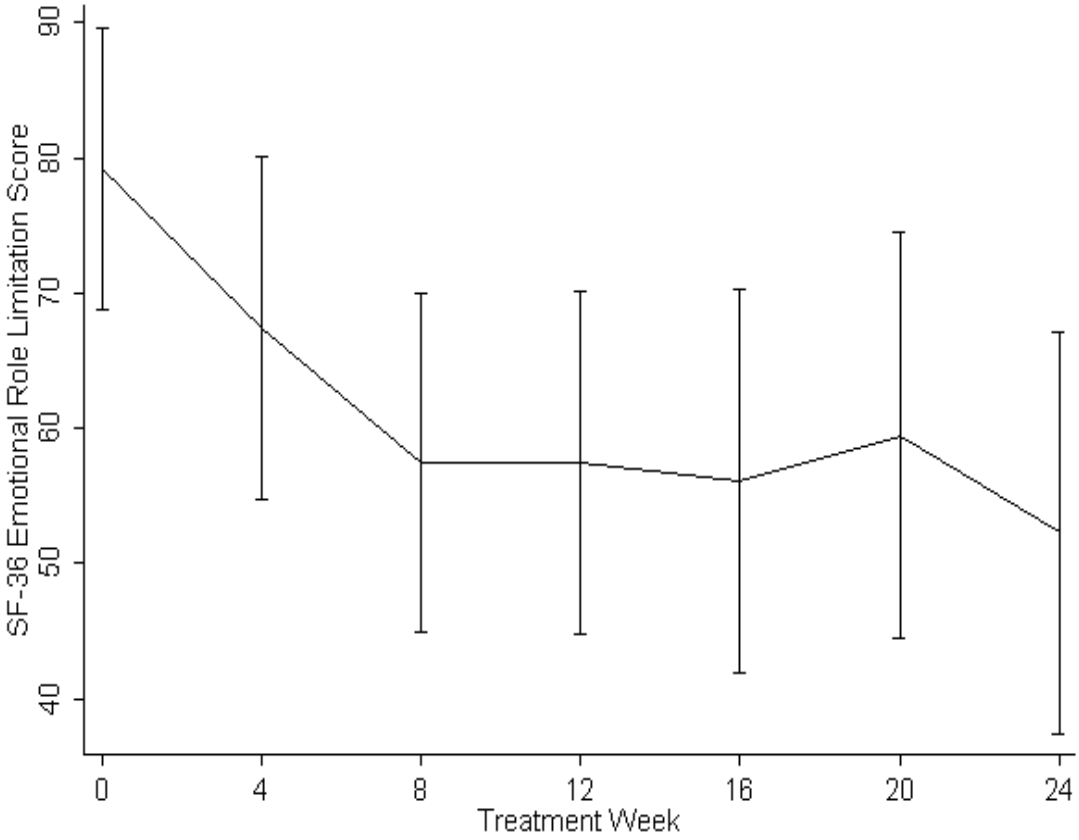


Figure 3.7 Changes in mean emotional role limitation scores during IFN-α treatment

Changes in mean scores on the emotional role limitation dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

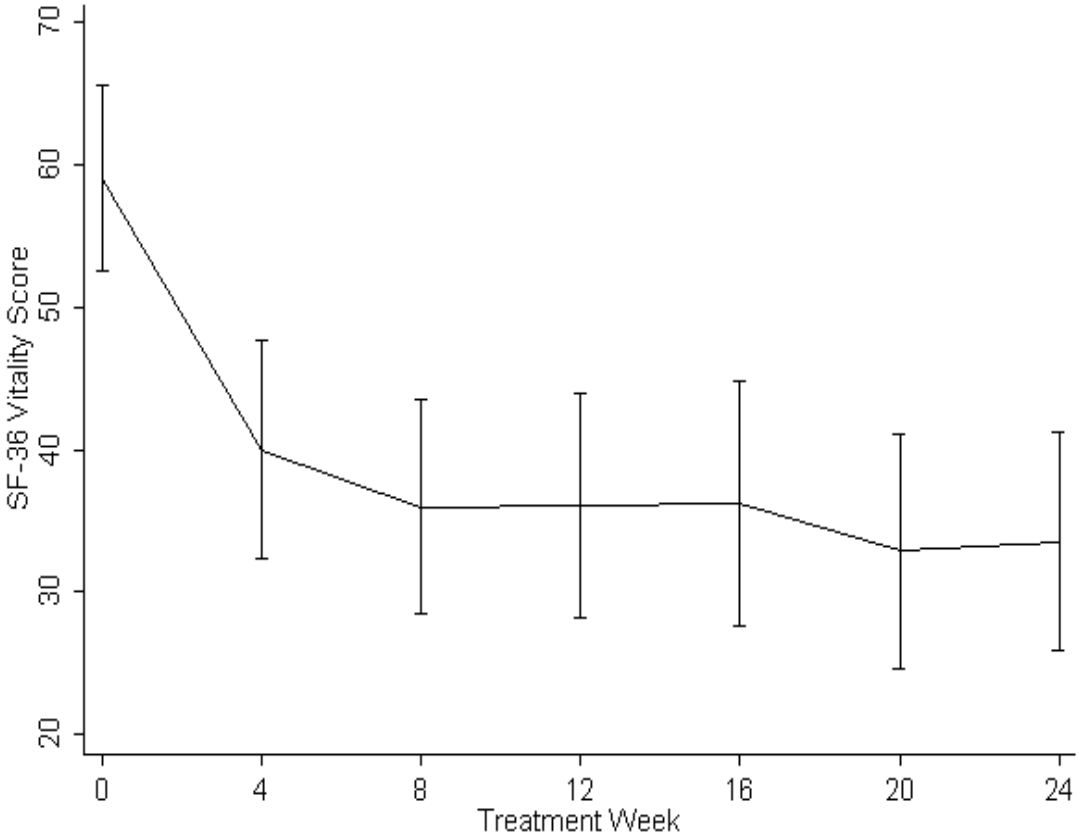


Figure 3.8 Changes in mean vitality scores during IFN-α treatment

Changes in mean scores on the vitality dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

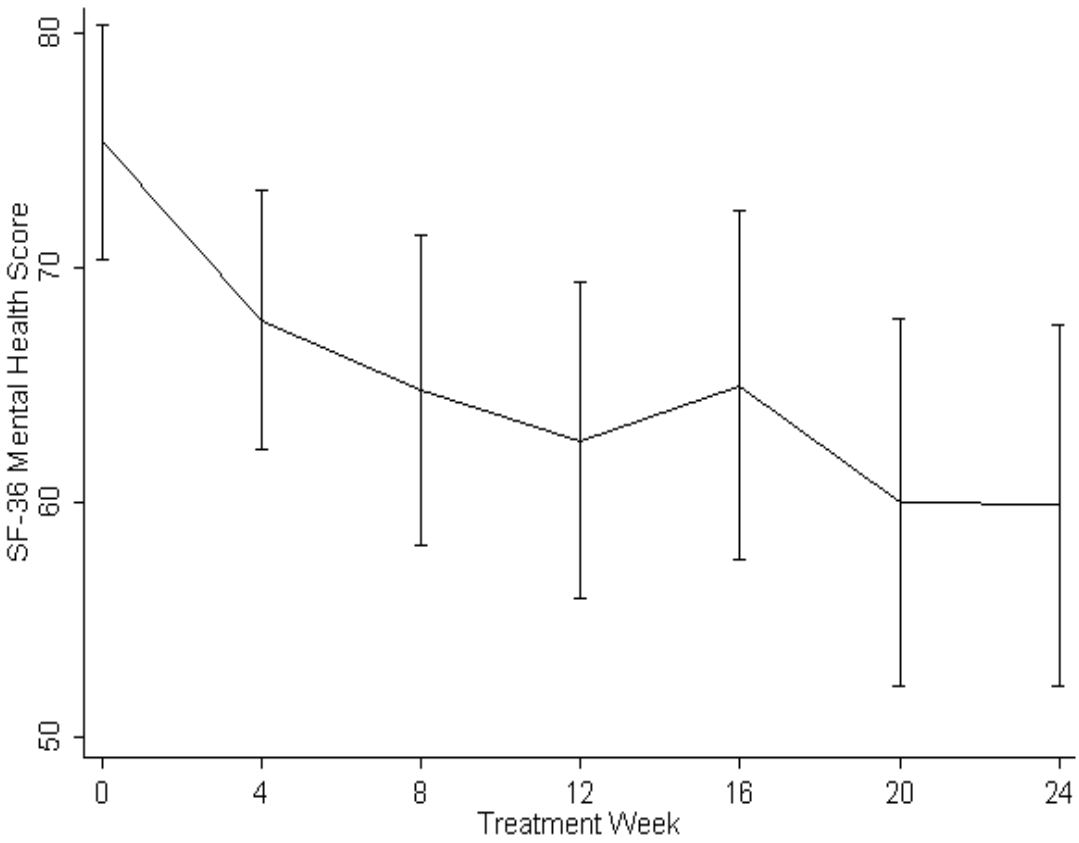


Figure 3.9 Changes in mean mental health scores during IFN-α treatment

Changes in mean scores on the mental health dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

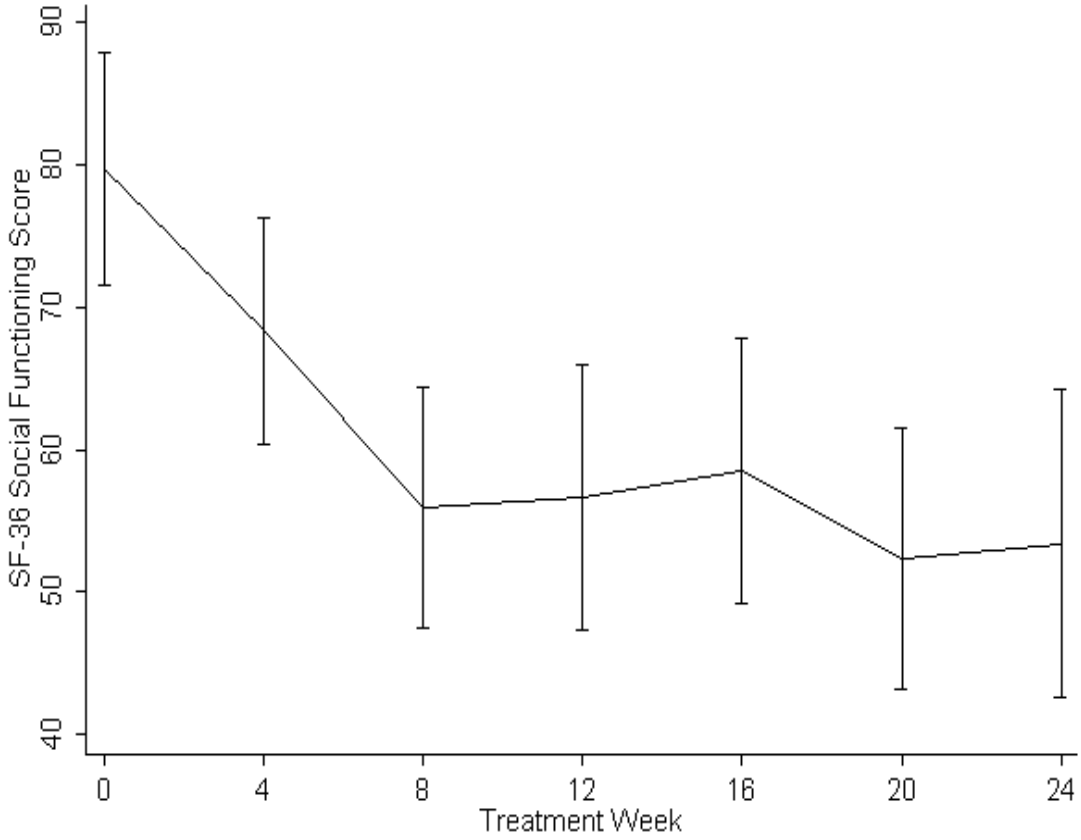


Figure 3.10 Changes in mean social functioning scores during IFN-α treatment

Changes in mean scores on the social functioning dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

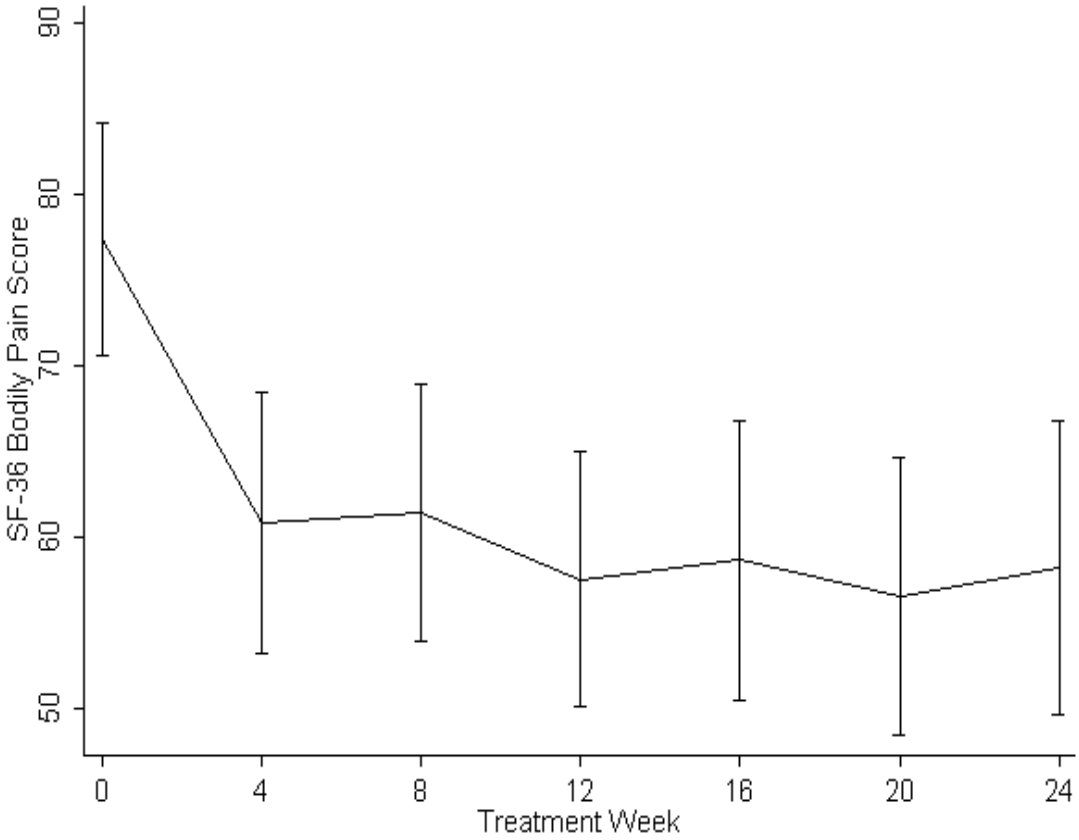


Figure 3.11 Changes in mean bodily pain scores during IFN-α treatment

Changes in mean scores on the bodily pain dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

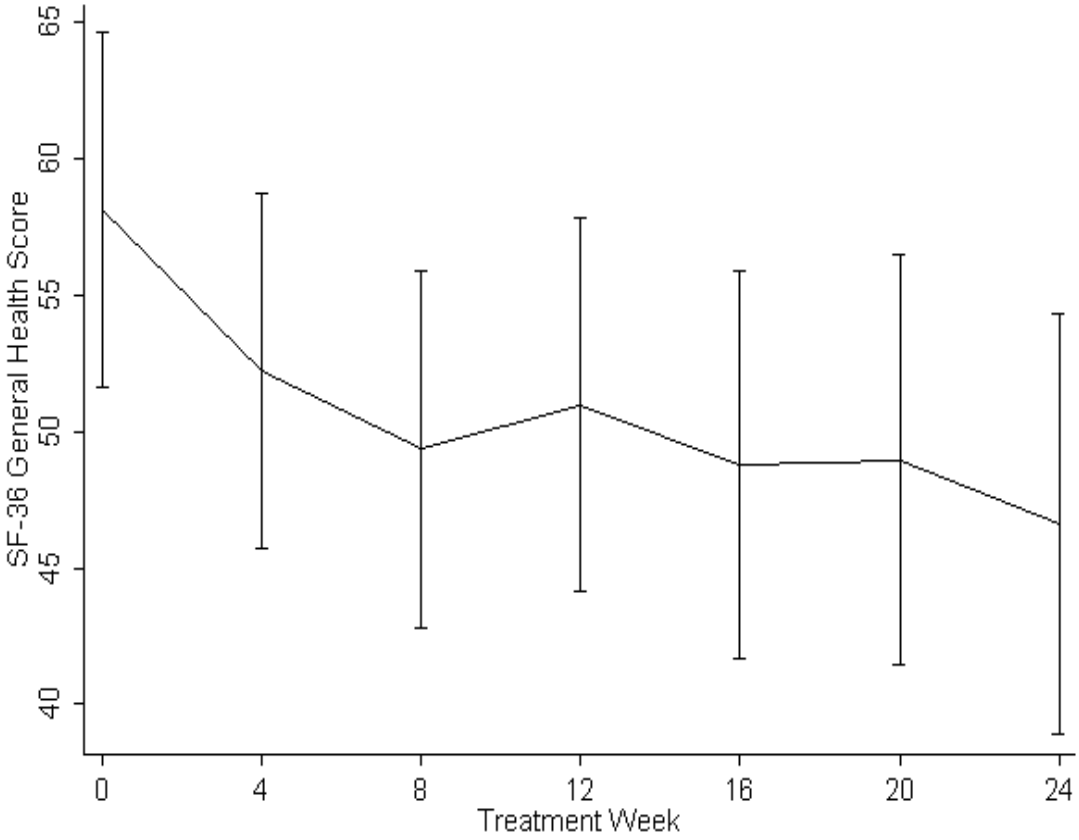


Figure 3.12 Changes in mean general health scores during IFN-α treatment

Changes in mean scores on the general health dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment (*n* ranging from 37-48).

As shown in Table 3.4, baseline scores of depression, fatigue, stress and anxiety were all highly correlated with each other. I also examined the relationship between baseline SF-36 scores and the baseline scores of depression, fatigue, stress and anxiety. As shown in Table 3.5, baseline scores for all 8 dimensions of the SF-36 were significantly, negatively correlated with baseline scores of depression, fatigue and stress. Only 4 out of the 8 dimensions of the SF-36 were significantly, negatively correlated with baseline anxiety scores. Specifically, these were emotional role limitation, vitality, mental health, social functioning (Coefficient=-0.4, $p=0.005$; Coefficient=-0.5, $p<0.001$; Coefficient=-0.8, $p<0.001$ and Coefficient=-0.4 $p=0.013$). There were no significant correlations between baseline scores of the physical functioning, physical role limitation, bodily pain or general health dimensions of the SF-36 and baseline anxiety scores ($p=1.0$, $p=0.3$, $p=0.4$ and $p=0.1$ respectively).

Table 3.4 The relationship between baseline scores of depression, fatigue, stress and anxiety

	Baseline scores			
Baseline scores	**IDS**	**CFQ**	**PSS**	**HADS-A**
IDS		r=0.7 **p<0.001**	r=0.7 **p<0.001**	r=0.8 **p<0.001**
CFQ	r=0.7 **p<0.001**		r=0.6 **p<0.001**	r=0.6 **p<0.001**
PSS	r=0.7 **p<0.001**	r=0.6 **p<0.001**		r=0.7 **p<0.001**
HADS-A	r=0.7 **p<0.001**	r=0.6 **p<0.001**	r=0.7 **p<0.001**	

Correlation analyses between baseline scores on the Inventory of Depressive Symptomatology (IDS), Chalder Fatigue Questionnaire (CFQ), Perceived Stress Scale (PSS) and the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n* ranging from 45-48).

Table 3.5 The relationship between baseline scores for the 8 dimensions of the SF-36 and the baseline scores of depression, fatigue, stress and anxiety

	Baseline scores			
Baseline scores	**IDS**	**CFQ**	**PSS**	**HADS-A**
Physical functioning	r=-0.4 **p=0.013**	r=-0.4 **p=0.017**	r=-0.4 **p=0.002**	r=<-0.01 p=1.0
Physical role limitation	r=-0.5 **p=0.001**	r=-0.4 **p=0.005**	r=-0.4 **p=0.001**	r=-0.2 p=0.3
Emotional role limitation	r=-0.5 **p<0.001**	r=-0.5 **p=0.001**	r=-0.4 **p=0.004**	r=-0.4 **p=0.005**
Vitality	r=-0.6 **p<0.001**	r=-0.6 **p<0.001**	r=-0.6 **p<0.001**	r=-0.5 **p<0.001**
Mental health	r=-0.7 **p<0.001**	r=-0.5 **p<0.001**	r=-0.6 **p<0.001**	r=-0.8 **p<0.001**
Social functioning	r=-0.5 **p<0.001**	r=-0.3 **p=0.041**	r=-0.6 **p<0.001**	r=-0.4 **p=0.013**
Bodily pain	r=-0.4 **p=0.004**	r=-0.4 **p=0.004**	r=-0.4 **p=0.005**	r=-0.1 p=0.4
General health	r=-0.4 **p=0.003**	r=-0.4 **p=0.007**	r=-0.5 **p<0.001**	r=-0.3 p=0.1

Correlation analyses between baseline scores on the 8 dimensions of the SF-36, and baseline scores on the Inventory of Depressive Symptomatology (IDS), Chalder Fatigue Questionnaire (CFQ), Perceived Stress Scale (PSS) and the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n* ranging from 45-48).

3.1.3 Biological changes during IFN-α treatment

As the final part of the first aim of this thesis, I prospectively monitored the impact of IFN-α treatment on three biological systems; HPA axis function, tryptophan and kynurenine pathway and PUFA levels. Furthermore, I also investigated gene expression changes.

3.1.3.1 Cortisol

Nineteen patients completed cortisol collection at baseline and 13 patients completed cortisol collection at treatment week 24 (end of treatment). To investigate changes in cortisol levels during IFN-α treatment, I firstly looked at the cortisol awakening response at baseline and at treatment week 24. These data are presented in Figure 3.13 and Figure 3.14. To further investigate the cortisol awakening response, I then calculated the area under the curve of the increase (AUCi) of the cortisol awakening response, considering cortisol levels at 0 minutes, 15 minutes, 30 minutes, and 60 minutes after awakening. Changes in the AUCi of the cortisol awakening response from baseline to treatment week 24 of IFN-α treatment are shown in Figure 3.15. There is a decrease in the average cortisol awakening response from baseline to treatment week 24, however, this effect of treatment was not significant (p=0.5).

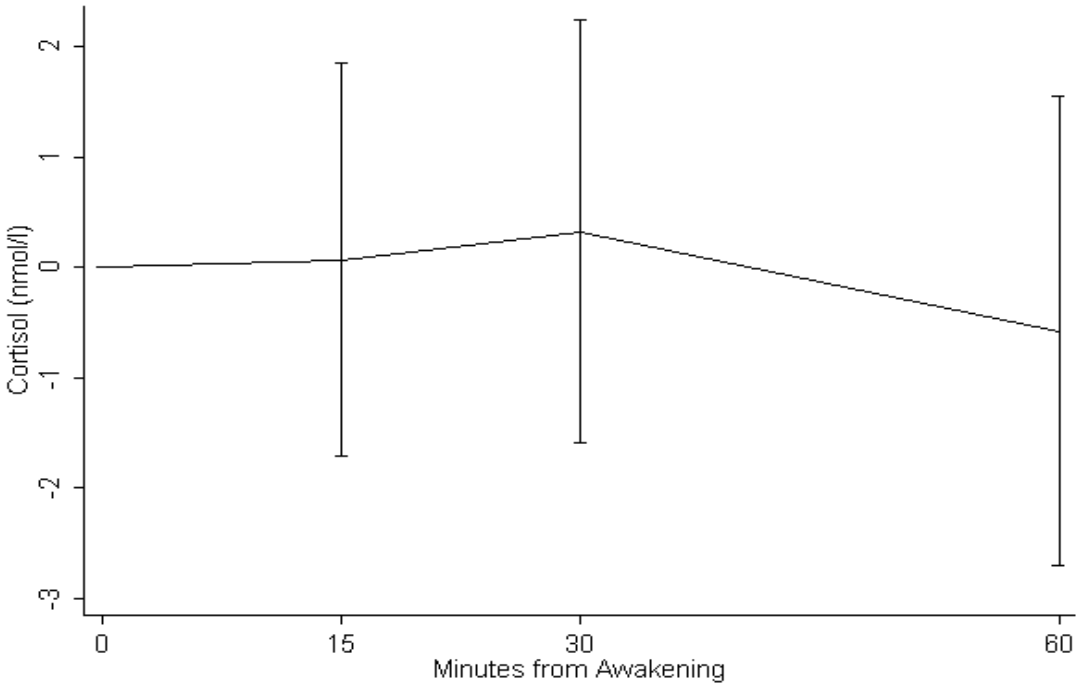


Figure 3.13 The cortisol awakening response at baseline (*n*=17)

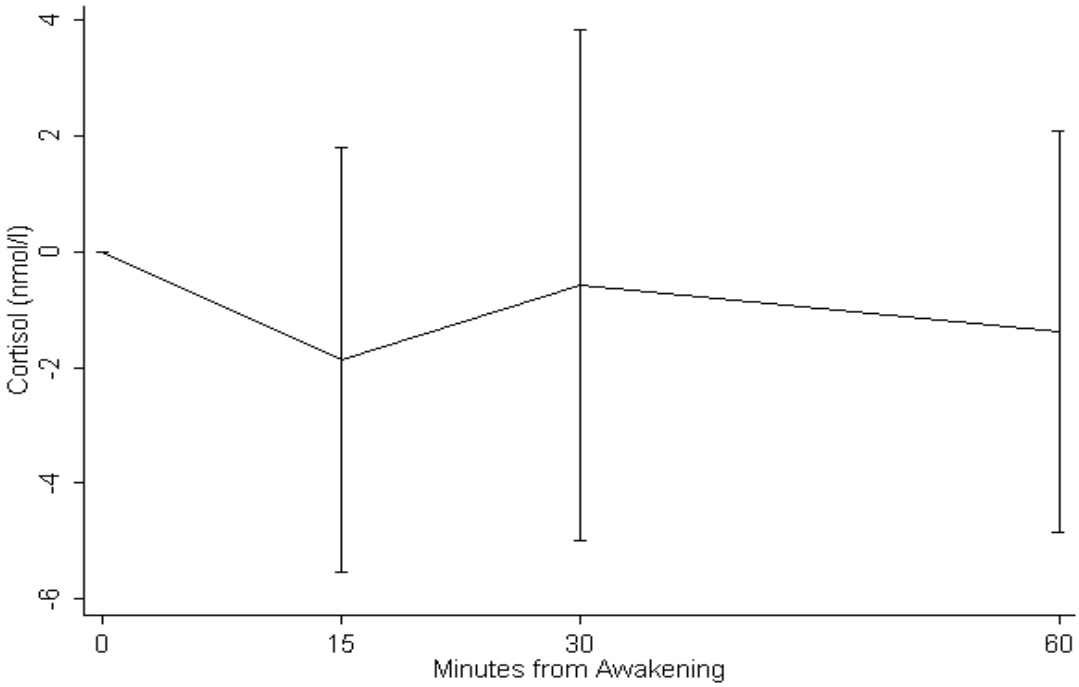


Figure 3.14 The cortisol awakening response at treatment week 24 of IFN-α treatment (*n*=11)

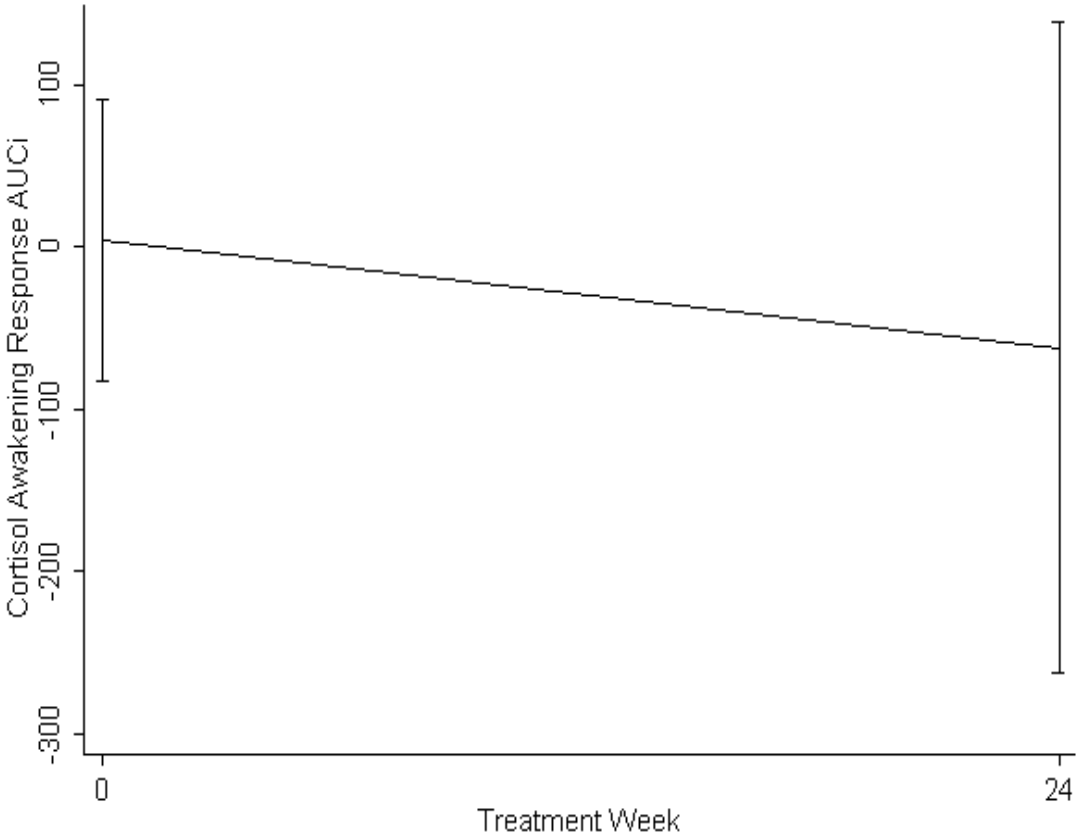


Figure 3.15 Changes in the area under the curve of the increase (AUCi) of the cortisol awakening response from baseline to treatment week 24 of IFN-α treatment (*n*=11)

I also investigated the changes in cortisol levels during the day. I firstly looked at the changes in the raw values of cortisol at 0 minutes (awakening), noon and 8pm from baseline to treatment week 24 of IFN-α treatment. These data are shown in Figure 3.16 and Figure 3.17. There was no significant effect of treatment week on cortisol levels at either noon or 8pm ($p=0.4$ and $p=0.3$). To further investigate the cortisol levels during the day, I then calculated the area under the curve (AUC) of cortisol levels at 0 minutes (awakening), at noon and at 8pm. Changes in the AUC of cortisol during the day from baseline to treatment week 24 of IFN-α treatment are shown in Figure 3.18. There is an increase in the AUC of cortisol during the day from baseline to treatment week 24, however, this effect of treatment week was not significant ($p=0.6$).

Finally, I investigated the relationship between baseline cortisol levels (AUCi of the cortisol awakening response and AUC of cortisol during the day) and baseline scores of depression, fatigue, stress and anxiety. Neither baseline cortisol awakening response, nor cortisol levels during the day were significantly correlated with baseline depression, fatigue, stress or anxiety scores. These data are presented in Table 3.6.

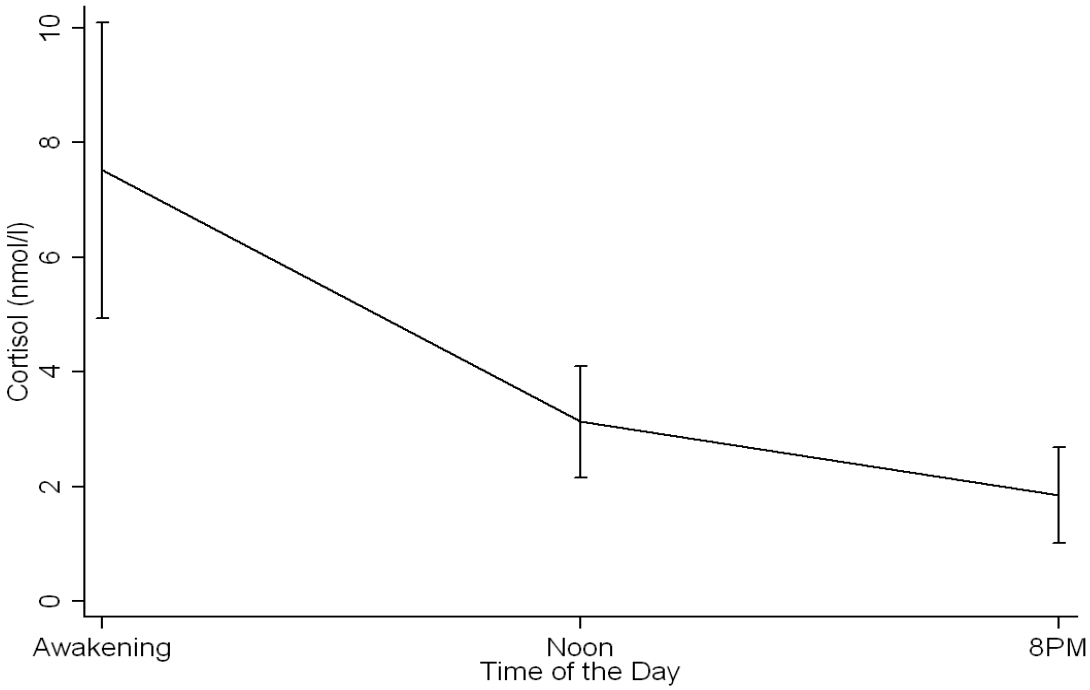


Figure 3.16 Cortisol levels during the day at baseline (*n*=19)

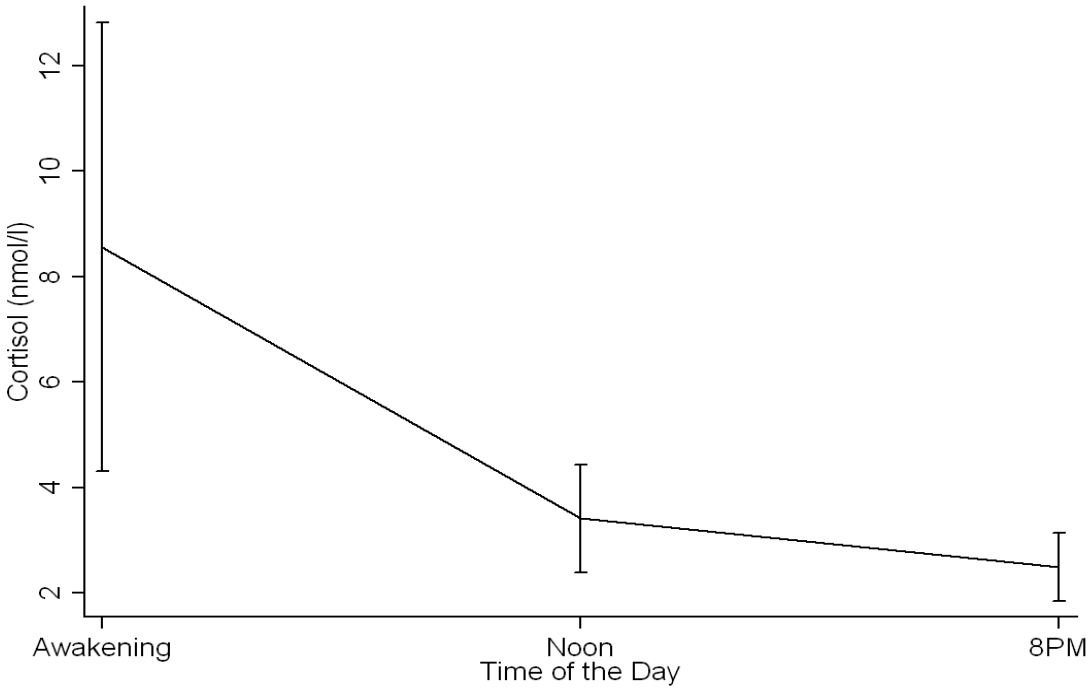


Figure 3.17 Cortisol levels during the day at treatment week 24 of IFN-α treatment (*n*=10)

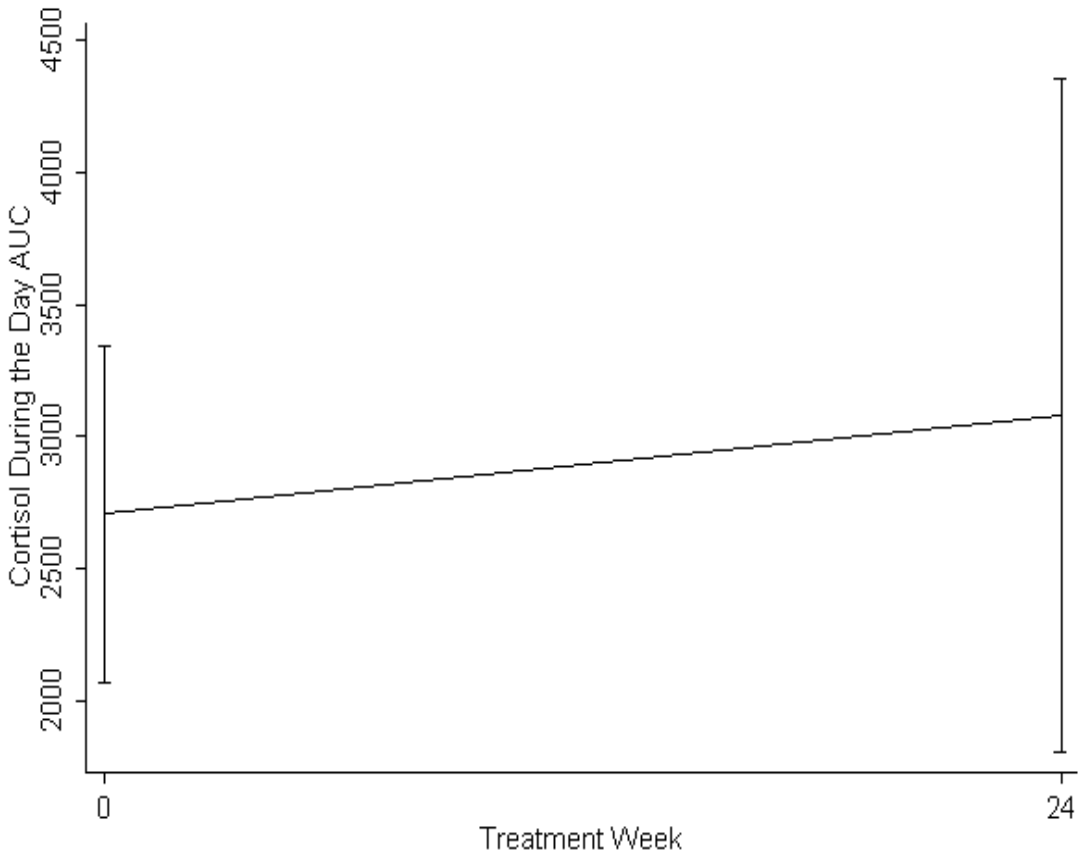


Figure 3.18 Changes in the area under the curve (AUC) of cortisol during the day from baseline to treatment week 24 of IFN-α treatment (*n*=10)

Table 3.6 The relationship between baseline cortisol levels and baseline depression, fatigue, stress and anxiety scores

	Baseline scores			
Baseline levels	**IDS**	**CFQ**	**PSS**	**HADS-A**
Cortisol awakening response (AUCi)	r=0.1 p=0.6	r=0.2 p=0.6	r=0.3 p=0.3	r=0.3 p=0.2
Cortisol during the day (AUC)	r=0.2 p=0.4	r=0.4 p=0.1	r=-0.01 p=1.0	r=-0.05 p=0.8

Correlation analyses between the baseline area under the curve of the increase (AUCi) of the cortisol awakening response and the baseline area under the curve (AUC) of cortisol during the day, with baseline scores on the on the Inventory of Depressive Symptomatology (IDS), Chalder Fatigue Questionnaire (CFQ), Perceived Stress Scale (PSS) and the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n* ranging from 17-19).

3.1.3.2 Kynurenine and Tryptophan pathway

Kynurenine and tryptophan pathway metabolites were measured in 38 patients at baseline, treatment week 8 and treatment week 24 (end of treatment). Changes in the levels of kynurenine and tryptophan pathway metabolites during IFN-α treatment are shown in Figure 3.19-Figure 3.23. Tryptophan levels decreased during IFN-α treatment, with a significant effect of treatment week (Coefficient=-0.04, p=0.054). Moreover, this decrease in tryptophan levels was accompanied by increased kynurenine levels, with a significant effect of treatment week on increasing kynurenine levels (Coefficient=3.1, p=0.003).

Levels of three-hydroxykynurenine also increased during IFN-α treatment, however this was not significant (Coefficient=0.1, p=0.1). Levels of the neuroprotective metabolite; kynurenic acid, decreased during IFN-α treatment with a significant effect of treatment week (Coefficient=-0.04, p=0.0045). Finally, the ratio of kynurenine to tryptophan increased during IFN-α treatment with a significant effect of treatment week (Coefficient=0.4, p=0.001).

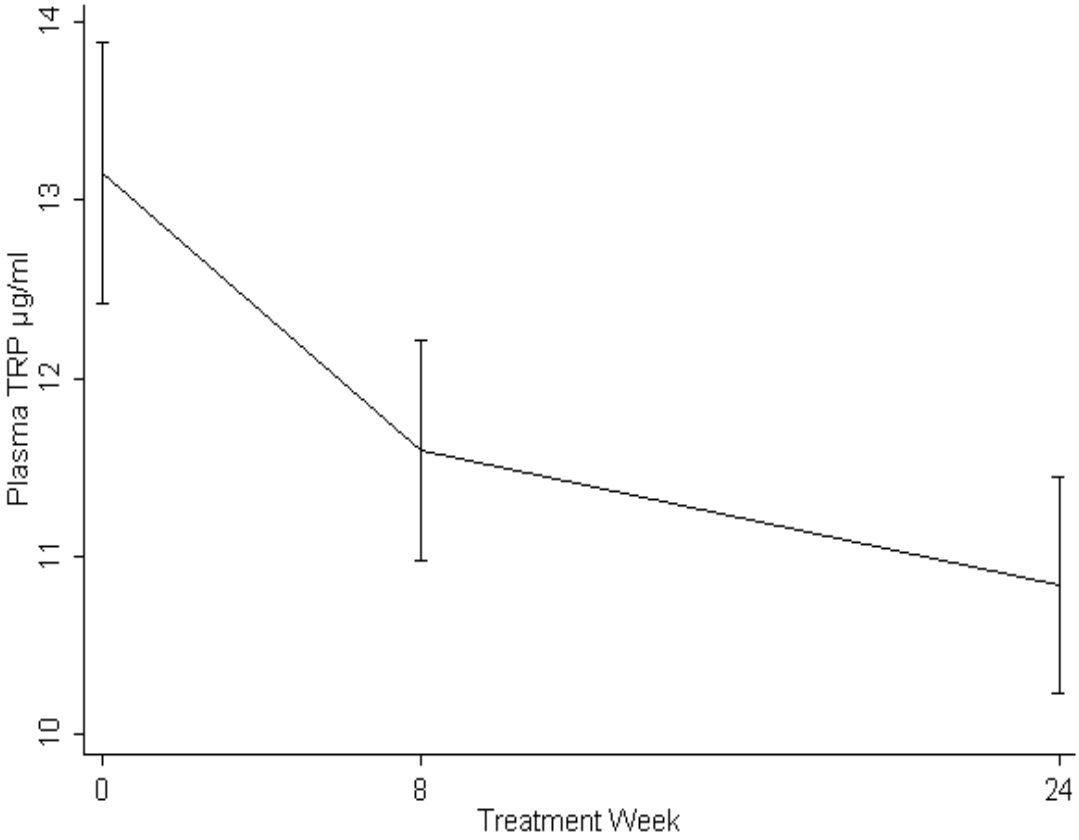


Figure 3.19 Changes in tryptophan levels during IFN-α treatment

Changes in plasma levels of tryptophan (TRP) across the 24 weeks of treatment (*n* ranging from 28-38).

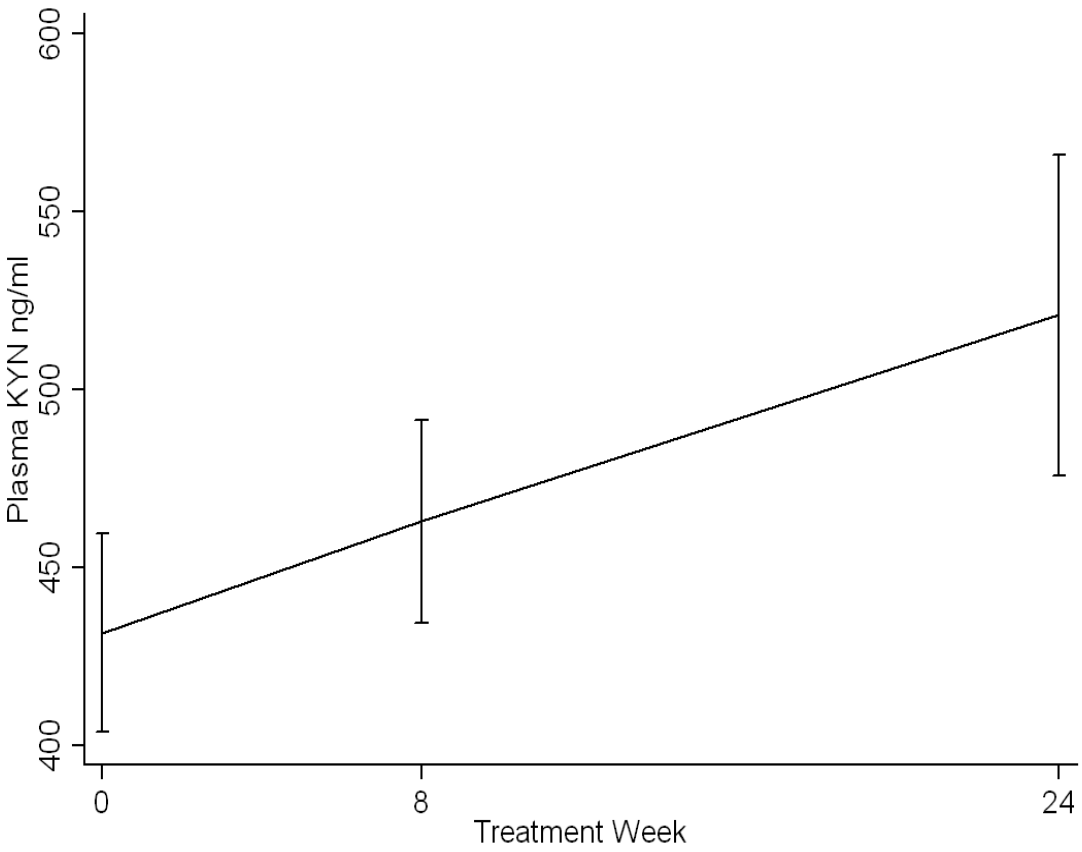


Figure 3.20 Changes in kynurenine levels during IFN-α treatment

Changes in plasma levels of kynurenine (KYN) across the 24 weeks of treatment (*n* ranging from 28-38).

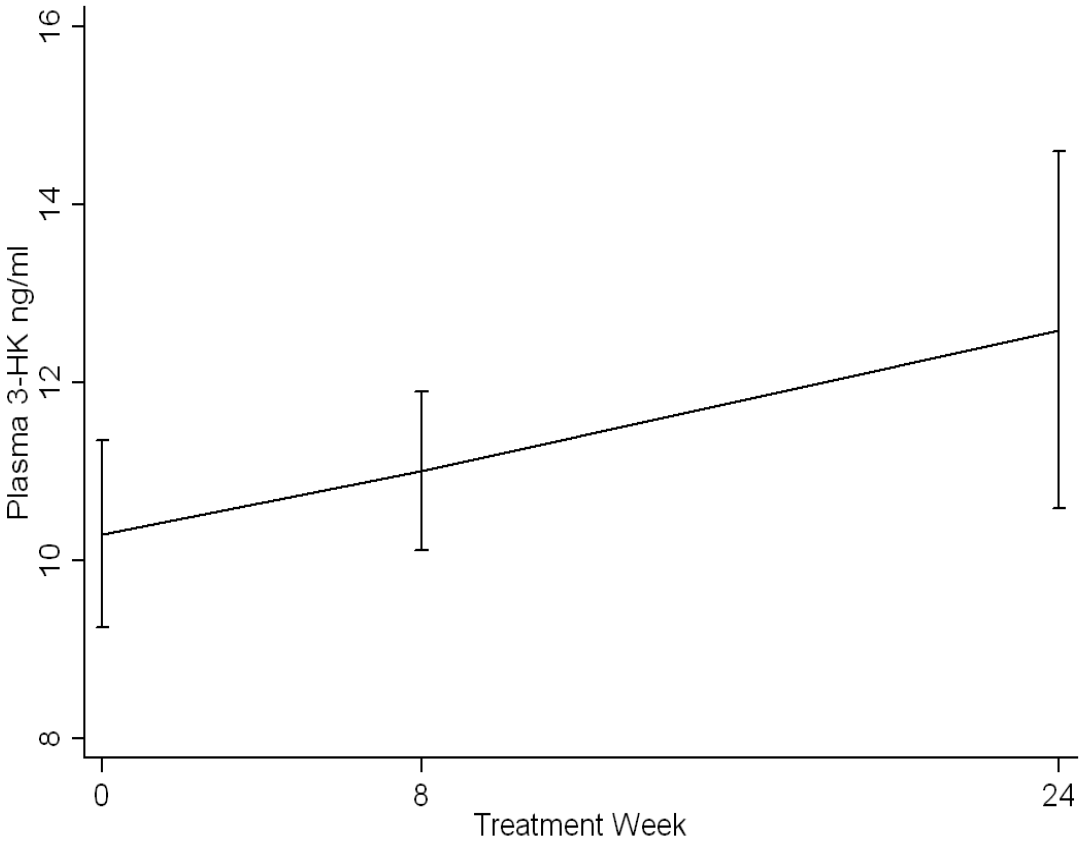


Figure 3.21 Changes in 3-hydroxykynurenine levels during IFN-α treatment

Changes in plasma levels of 3-hydroxykynurenine (3-HK) across the 24 weeks of treatment (*n* ranging from 28-38).

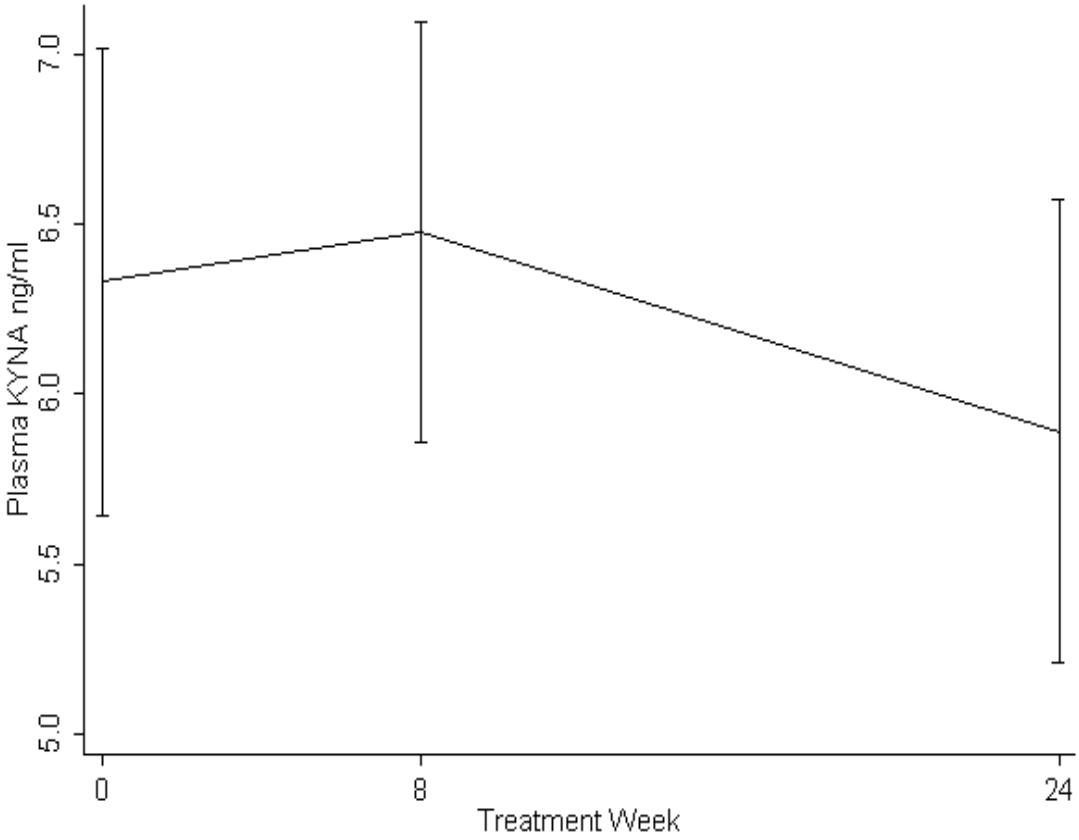


Figure 3.22 Changes in kynurenic acid levels during IFN-α treatment

Changes in plasma levels of kynurenic acid (KYNA) across the 24 weeks of treatment (*n* ranging from 28-38).

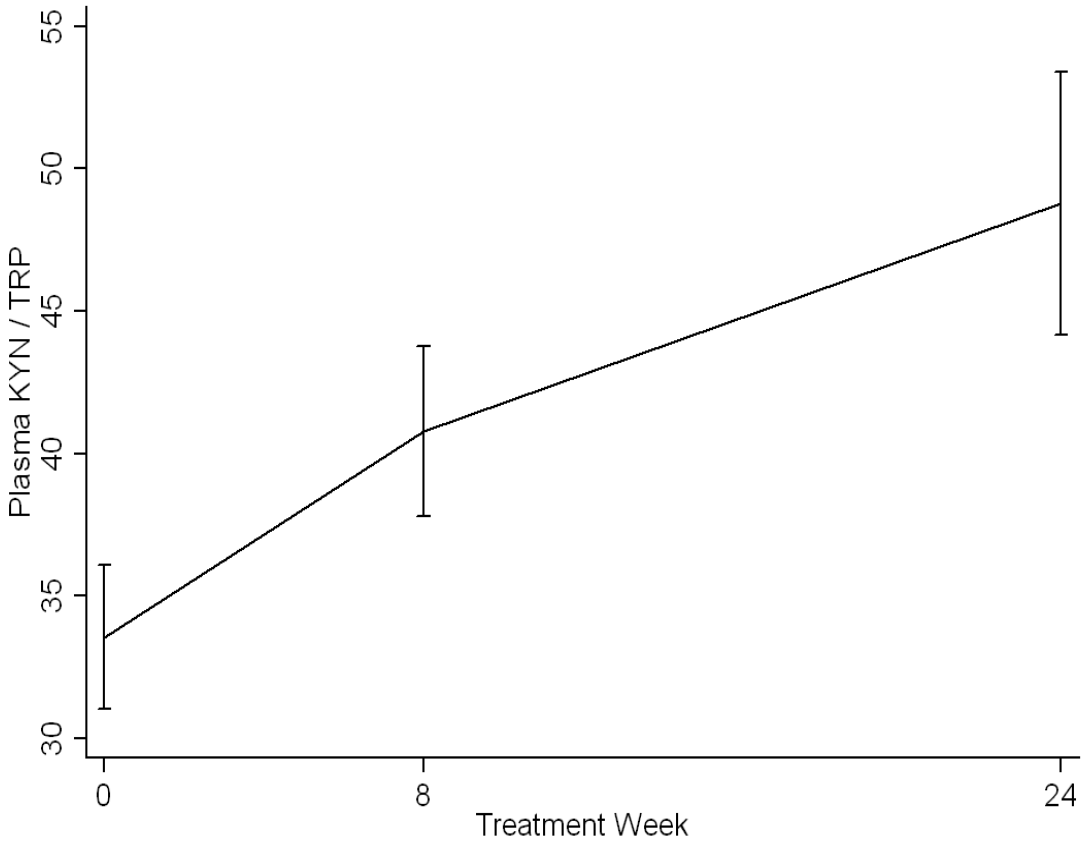


Figure 3.23 Changes in the kynurenine/tryptophan ratio during IFN-α treatment

Changes in the kynurenine/tryptophan ratio (KYN/TRP) across the 24 weeks of treatment (*n* ranging from 28-38).

The relationship between baseline levels of kynurenine and tryptophan pathway metabolites and baseline scores of depression, fatigue, stress and anxiety are presented in Table 3.7. Baseline levels of tryptophan, kynurenine, 3-hydroxykynurenine and the kynurenine/tryptophan ratio were not significantly correlated with baseline scores of any of the four outcome measures. However, baseline kynurenic acid levels were significantly negatively correlated with baseline scores for both fatigue and stress ($r=-0.4$, $p=0.031$ and $r=-0.4$, $p=0.023$ respectively). These significant correlations can be seen in Figure 3.24 and Figure 3.25).

Table 3.7 The relationship between baseline kynurenine and tryptophan pathway metabolites levels and baseline depression, fatigue, stress and anxiety scores

	Baseline scores			
Baseline levels	**IDS**	**CFQ**	**PSS**	**HADS-A**
Tryptophan ug/l	r=-0.3 p=0.1	r=0.1 p=0.5	r=0.02 p=0.9	r=-0.1 p=0.5
Kynurenine ng/l	r=-0.1 p=0.4	r=0.03 p=0.9	r=-0.1 p=0.4	r=-0.05 p=0.8
3-HK ng/l	r=-0.02 p=0.9	r=-0.02 p=0.9	r=-0.09 p=0.6	r=<-0.01 p=1.0
Kynurenic acid ng/l	r=-0.2 p=0.2	r=-0.4 **p=0.031**	r=-0.4 **p=0.023**	r=-0.3 p=0.1
Kynurenine/ Tryptophan ratio	r=0.1 p=0.6	r=-0.03 p=0.8	r=-0.1 p=0.6	r=0.02 p=0.9

Correlation analyses between baseline levels of plasma tryptophan, plasma kynurenine, plasma 3-hydroxykynurenine (3-HK), plasma kynurenic acid and the ratio of kynurenine to tryptophan, with baseline scores on the on the Inventory of Depressive Symptomatology (IDS), Chalder Fatigue Questionnaire (CFQ), Perceived Stress Scale (PSS) and the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n* ranging from 37-38).

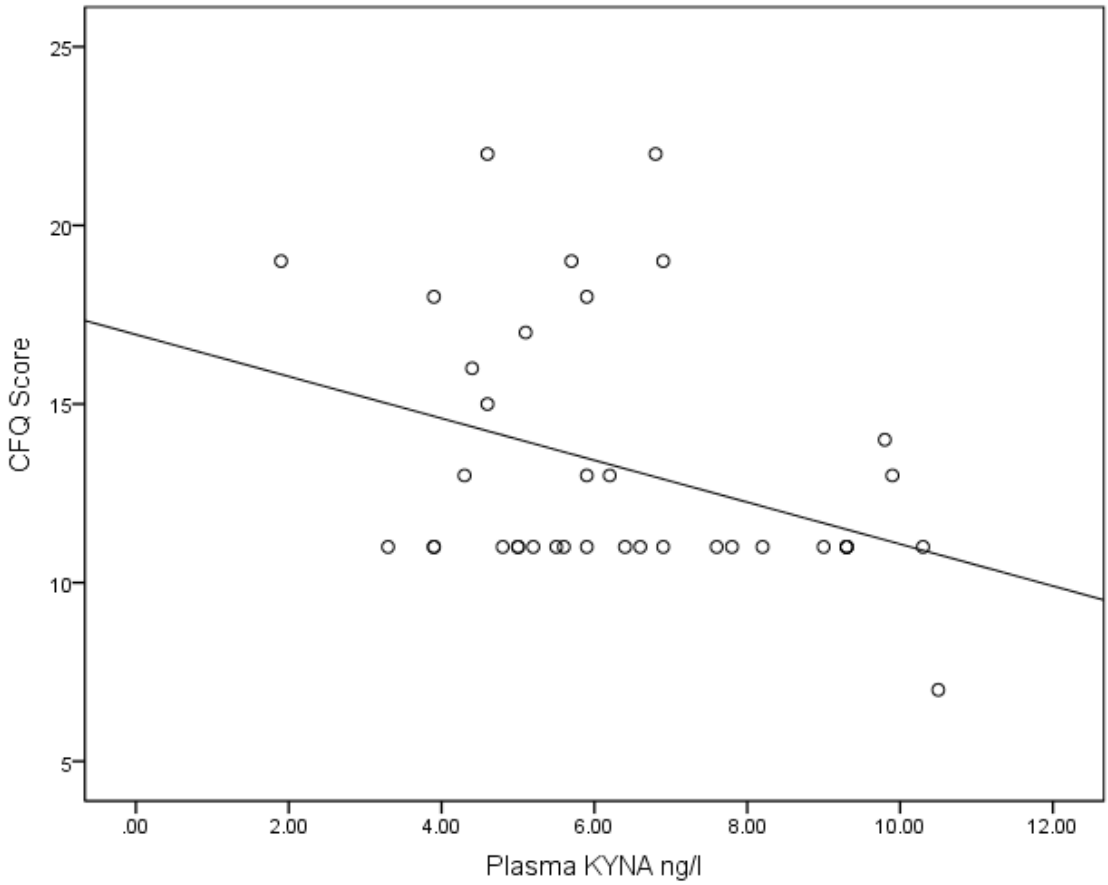


Figure 3.24 The correlation between baseline levels of KYNA and baseline CFQ scores.

A scatterplot of baseline levels of plasma kynurenic acid (KYNA) and baseline scores on the Chalder Fatigue Questionnaire (CFQ) (*n*=37).

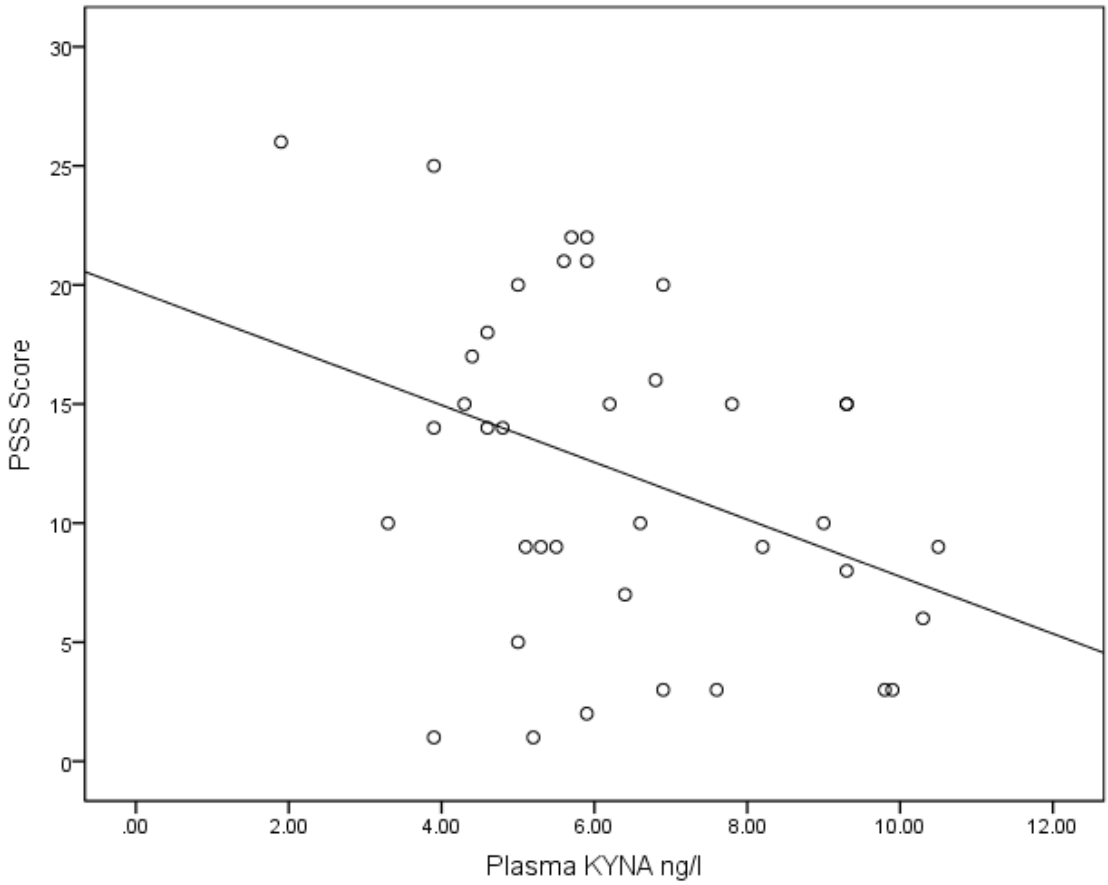


Figure 3.25 The correlation between baseline levels of KYNA and baseline PSS scores.

A scatterplot of baseline levels of plasma kynurenic acid (KYNA) and baseline scores on the Percevied Stress Scale (PSS) (*n*=38).

3.1.3.3 Polyunsaturated fatty acids (PUFAs)

PUFA levels were measured in 45 patients at all time points. Changes in omega-3 and omega-6 PUFAs during IFN-α treatment are shown in Figure 3.26-Figure 3.31. Eicosapentaenoic acid (EPA) average levels decreased slightly between baseline and treatment week 12; however, there was no significant effect of treatment week ($p=0.9$). Docosahexaenoic acid (DHA) levels also decreased during IFN-α treatment, and there was a significant effect of treatment week (Coefficient=-0.01, $p=0.033$). Levels of the omega-3 PUFA alpha-linolenic acid (ALA) increased between baseline and treatment week 12, and then decreased between treatment week 12 and treatment week 24. However, there was no significant effect of treatment week on changes in ALA levels ($p=0.4$). The two omega-6 PUFAs that were measured – arachidonic acid (AA) and linoleic acid (LA) - both decreased during IFN-α treatment. There was a significant effect of treatment week on decreasing AA levels (Coefficient=-0.01, $p=0.017$). However, there was no significant effect of treatment week on changes in LA levels. Similarly, there was no significant effect of treatment week on the ratio of omega-6 to omega-3 PUFAs ($p=0.7$).

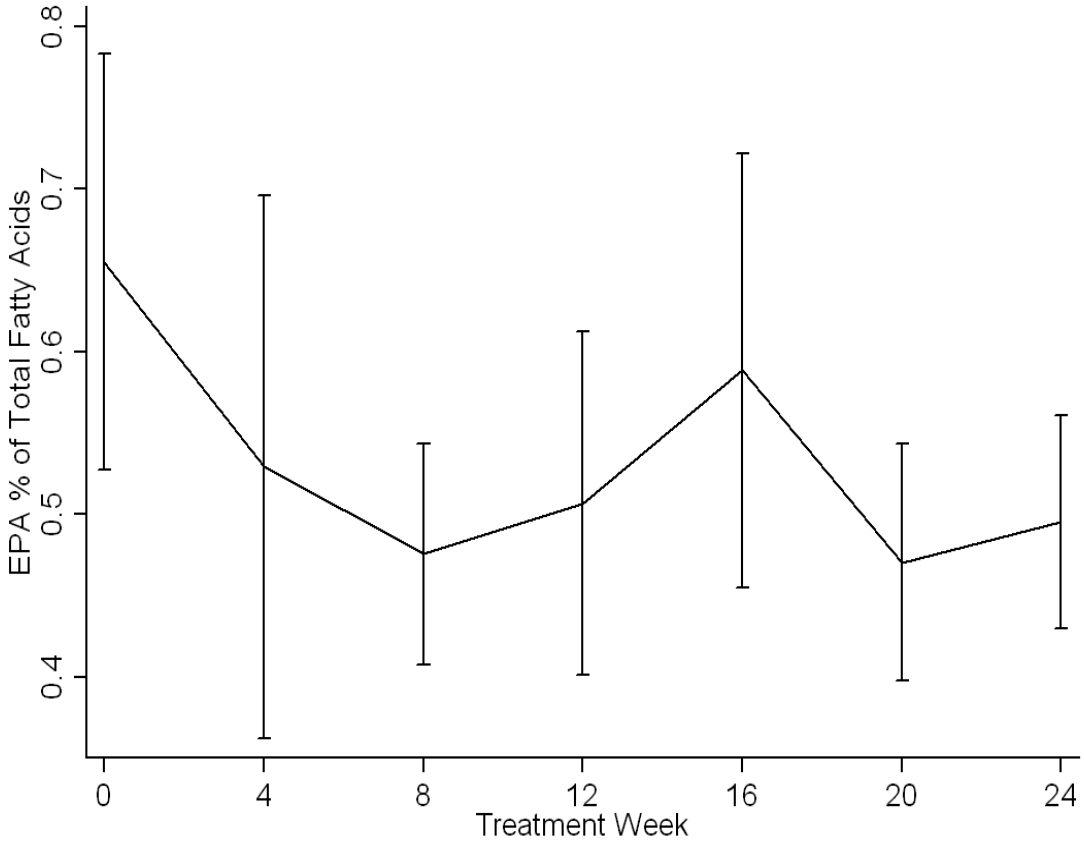


Figure 3.26 Changes in EPA levels during IFN-α treatment

Changes in plasma levels of the omega-3 PUFA, eicosapentaenoic acid (EPA) across the 24 weeks of treatment (*n* ranging from 34-48).

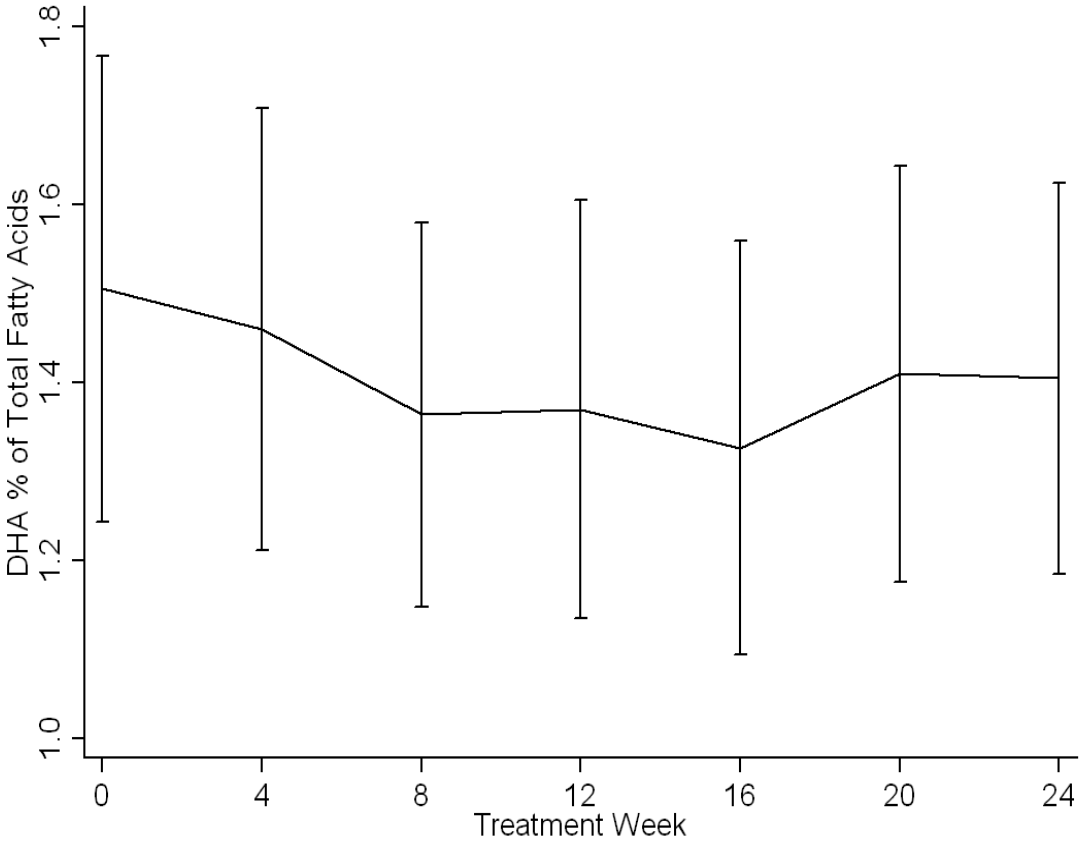


Figure 3.27 Changes in DHA levels during IFN-α treatment

Changes in plasma levels of the omega-3 PUFA, docosahexaenoic acid (DHA) across the 24 weeks of treatment (*n* ranging from 34-48).

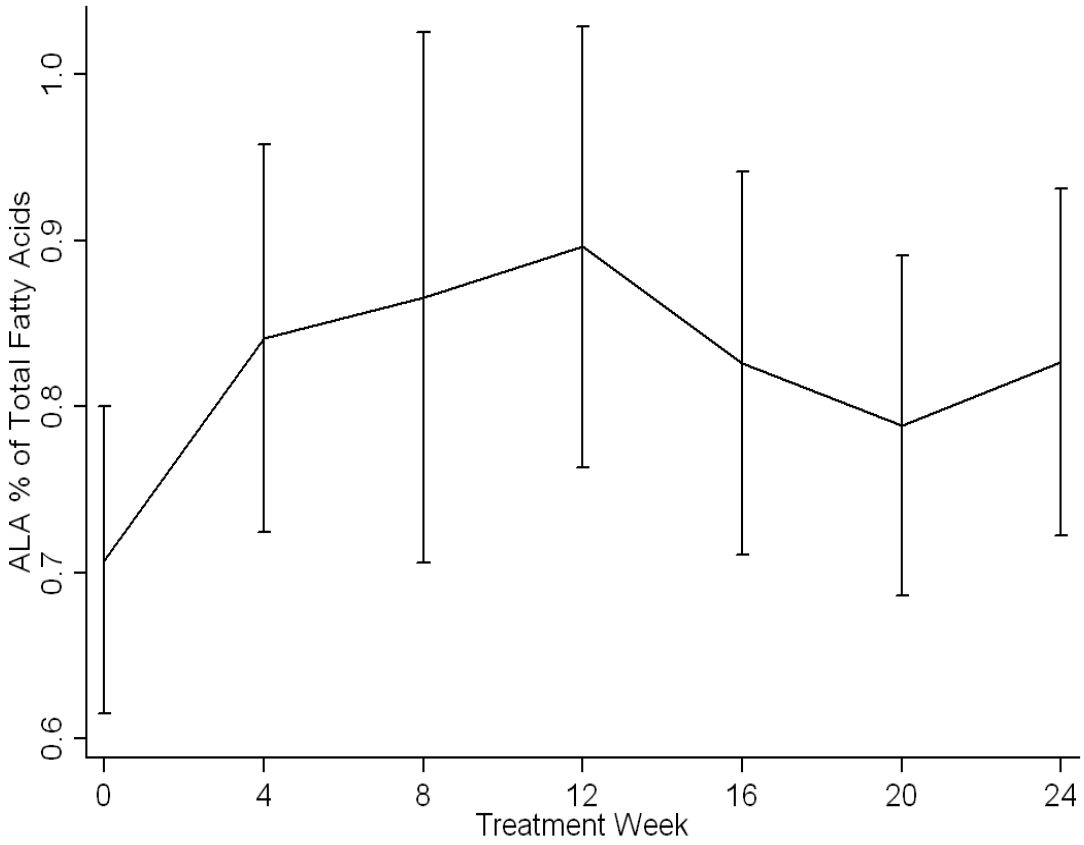


Figure 3.28 Changes in ALA levels during IFN-α treatment

Changes in plasma levels of the omega-3 PUFA, alpha-linolenic acid (ALA) across the 24 weeks of treatment (*n* ranging from 34-48).

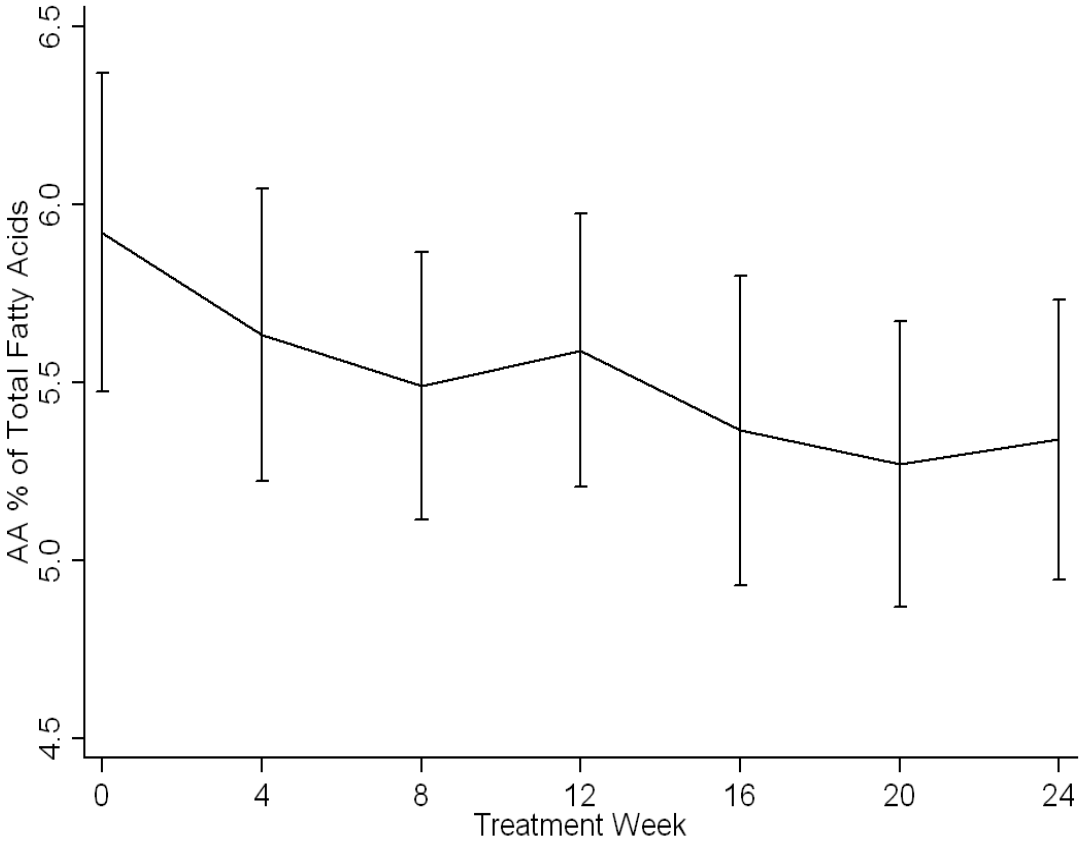


Figure 3.29 Changes in AA levels during IFN-α treatment

Changes in plasma levels of the omega-6 PUFA, arachidonic acid (AA) across the 24 weeks of treatment (*n* ranging from 34-48).

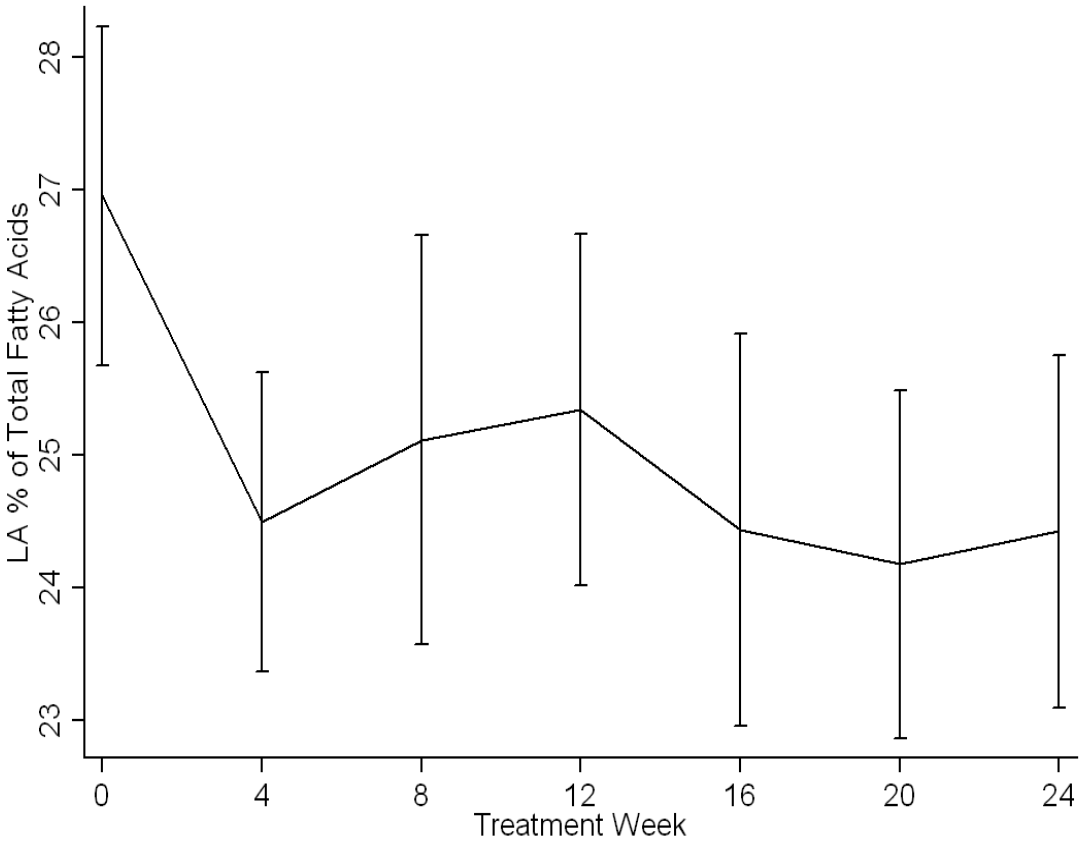


Figure 3.30 Changes in LA levels during IFN-α treatment

Changes in plasma levels of the omega-6 PUFA, linoleic acid (LA) across the 24 weeks of treatment (*n* ranging from 34-48).

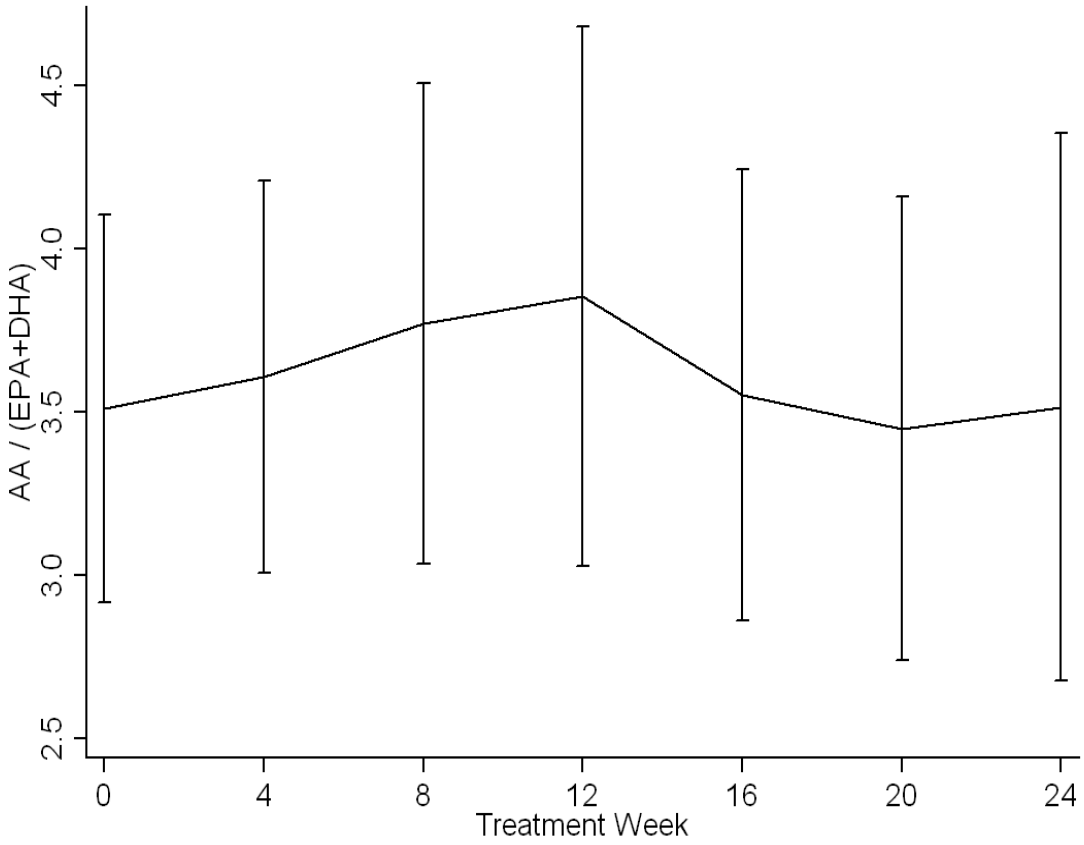


Figure 3.31 Changes in the AA/(EPA+DHA) ratio during IFN-α treatment

Changes in ratio of the omega-6 PUFA, arachidonic acid (AA), to the omega-3 PUFAs; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), across the 24 weeks of treatment (*n* ranging from 34-48).

The relationship between baseline PUFA levels and baseline scores of depression, fatigue, stress and anxiety are presented in Table 3.8. Only baseline levels of the omega-3 PUFA ALA were significantly negatively correlated with baseline anxiety scores ($r=-0.3$, $p=0.024$). This significant correlation can also be seen presented in Figure 3.32. There were no other significant correlations between baseline PUFA levels and baseline scores of depression, fatigue, stress or anxiety.

Table 3.8 The relationship between baseline PUFA levels and baseline depression, fatigue, stress and anxiety scores

	Baseline scores			
Baseline levels	**IDS**	**CFQ**	**PSS**	**HADS-A**
EPA	r=0.1 p=0.4	r=-0.1 p=0.7	r=0.1 p=0.4	r=0.04 p=0.8
DHA	r=0.04 p=0.8	r=-0.1 p=0.6	r=0.1 p=0.4	r=0.1 p=0.5
ALA	r=-0.2 p=0.3	r=-0.1 p=0.6	r=-0.01 p=1.0	r=-0.3 **p=0.024**
AA	r=0.2 p=0.3	r=-0.03 p=0.9	r=-0.1 p=0.5	r=0.1 p=0.5
LA	r=-0.1 p=0.5	r=-0.2 p=0.2	r=-0.2 p=0.3	r=-0.1 p=0.5
AA/(EPA+DHA) ratio	r=-0.03 p=0.8	r=-0.02 p=0.9	r=-0.3 p=0.1	r=-0.1 p=0.6

Correlation analyses between baseline levels of plasma eicosapentaenoic acid (EPA), plasma docosahexaenoic acid (DHA), plasma alpha-linolenic acid (ALA), plasma arachidonic acid (AA), plasma linoleic acid (LA) and the ratio of omega-6 to omega-3 PUFAs, with baseline scores on the on the Inventory of Depressive Symptomatology (IDS), Chalder Fatigue Questionnaire (CFQ), Perceived Stress Scale (PSS) and the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n* ranging from 43-44).

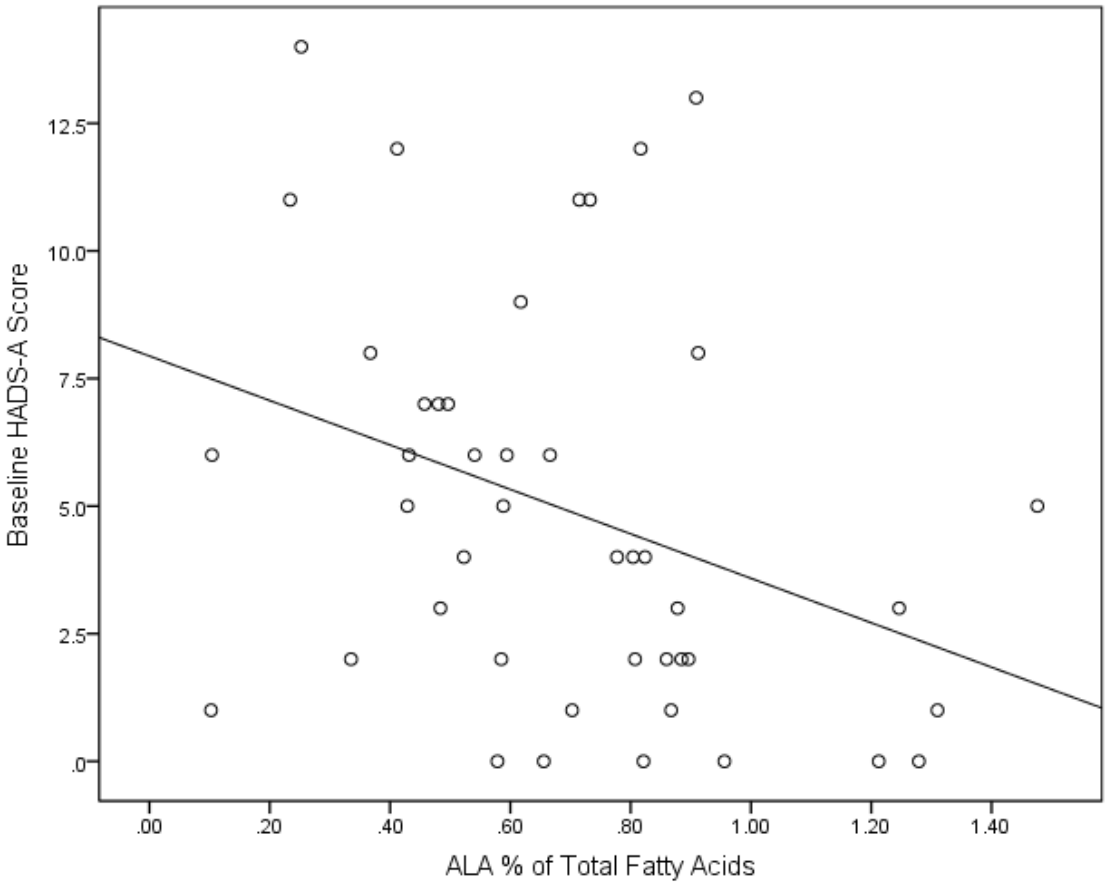


Figure 3.32 The correlation between baseline levels of ALA and baseline HADS-A scores

A scatterplot of baseline levels of plasma alpha-linolenic acid (ALA) and baseline scores on the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) (*n*=44).

3.1.3.4 Gene Expression

Gene expression using microarray was measured in all 48 patients at baseline and at treatment week 4. To investigate gene expression changes as a result of IFN-α treatment, I compared gene expression at treatment week 4 with gene expression at baseline. Firstly, I used a hypothesis-free approach, identifying genes with an absolute fold change of 1.4 and a p value cut-off of $p<0.05$. There were a total of 516 genes that were modulated by IFN-α treatment. These individual genes are not presented. However, pathway analysis revealed these genes belong to five pathways: mitogen-activated protein kinase (MAPK) signalling, calcium signalling, neurotrophin signalling, cancer and prostate cancer pathways.

Secondly, I used a hypothesis-driven approach and looked at changes in the expression of candidate genes, again comparing treatment week 4 with baseline. I chose genes belonging to 4 important and interlinked domains: genes related to tryptophan metabolism, genes related to PUFA metabolism, genes related to inflammation, and genes related to neuroplasticity. These data are presented in Table 3.9 and Table 3.10. Due to the fact that many of the genes had small fold changes (less than 1.1), fold change in these tables are presented to 2 decimal places. Of note, such low fold changes are likely to be false positives, are difficult to validate with real-time polymerase chain reaction (PCR) techniques and so are not considered as biologically relevant changes (Mutch et al., 2002, Ryan et al., 2010).

There were significant changes in the expression of kynureninase (KYNU) and tryptophan 2,3-dioxygenase (TDO2) as a result of IFN-α treatment ($p=0.009$

and p=0.030, respectively). Expression of KYNU was higher and expression of TDO2 was lower at treatment week 4 when compared to baseline. With regards to genes related to PUFA metabolism, there was significantly higher expression of Cyclooxygenase 2 (COX2), Cyclooxygenase 3 (COX3) and Phospholipase A2, group III (PLA2G3) at treatment week 4 when compared to baseline (p=0.047, p=0.004 and p=0.006, respectively). Furthermore, there was significantly higher expression of fatty acid desaturase 2 (FADS2) at treatment week 4 when compared to baseline (p=0.006), with an absolute fold change of 1.5.

Table 3.9 Differentially expressed candidate genes at treatment week 4 compared to baseline (*n*=45)

Gene Symbol	Gene Title	Fold Change	P Value
Tryptophan metabolism			
IDO1	Indoleamine 2,3-dioxygenase 1	-1.11	0.3
IDO2	Indoleamine 2,3-dioxygenase 2	-1.07	0.1
KMO	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	1.09	0.2
KYNU	Kynureninase	1.05	**0.009**
HAAO	3-hydroxyanthranilate 3,4-dioxygenase	-1.03	0.1
TDO2	Tryptophan 2,3-dioxygenase	-1.03	**0.030**
TPH1	Tryptophan hydroxylase 1	1.02	0.1
PUFA metabolism			
COX1	Cyclooxygenase 1	-1.09	0.5
COX2	Cyclooxygenase 2	-1.05	**0.047**
COX3	Cyclooxygenase 3	-1.20	**0.004**
FADS1	Fatty acid desaturase 1	1.13	0.2
FADS2	Fatty acid desaturase 2	1.53	**0.006**
PLA2G2A	Phospholipase A2, group II	1.04	0.1

Within the candidate genes involved in inflammation, there was significantly lower expression of interleukin 1 beta (IL-1B), interleukin 4 (IL-4), interleukin 6 receptor (IL-6R) and interleukin 28 beta (IL-28B) at treatment week 4 when compared with baseline ($p<0.001$, $p<0.001$, $p<0.001$ and $p=0.031$, respectively), but a significantly higher expression of interleukin 10 (IL-10) ($p=0.010$).

The only significant change in the expression of genes involved in neuroplasticity as a result of IFN-α treatment, was lower expression of nuclear receptor subfamily 3, group C, member 1 (NR3C1) ($p<0.001$), that is, of the glucocorticoid receptor (GR).

Table 3.10 Differentially expressed candidate genes at treatment week 4 compared to baseline (*n*=45)

Gene Symbol	Gene Title	Fold Change	P Value
Inflammation			
IL-1A	Interleukin 1, alpha	-1.01	0.5
IL-1B	Interleukin 1, beta	-1.41	**<0.001**
IL-1R1	Interleukin 1 receptor, type 1	-1.61	0.1
IL-2	Interleukin 2	-1.07	0.2
sIL-2R	Soluble interleukin 2 receptor	1.01	0.9
IL-4	Interleukin 4	-1.04	**<0.001**
IL-6	Interleukin 6	-1.02	0.8
IL-6R	Interleukin 6 receptor	-1.40	**<0.001**
IL-8	Interleukin 8	1.04	0.3
IL-10	Interleukin 10	1.12	**0.010**
IL-18	Interleukin 18	1.20	4.0
IL-28B	Interleukin 28, beta	-1.01	**0.031**
IFNG	Interferon, gamma	1.03	0.1
TGFB1	Transforming growth factor, beta 1	1.08	0.3
TNFA	Tumor necrosis factor, alpha	1.02	0.6
TRAF6	TNF receptor associated factor 6	1.12	0.1
Neuroplasticity			
BDNF	Brain-derived neurotrophic factor	-1.02	0.6
FKBP4	FK506 binding protein 4	1.01	0.1
FKBP5	FK506 binding protein 5	1.07	0.7
NR3C1	Nuclear receptor subfamily 3, group C, member 1	-1.10	**<0.001**
VEGFA	Vascular endothelial growth factor A	-1.04	0.2
VGF	VGF nerve growth factor inducible	-1.00	0.8

3.1.4 Risk of IFN-α-induced depression

In order to understand any risk factors associated with the development of IFN-α-induced depression, I investigated differences in the baseline characteristics of patients with and without IFN-α-induced depression. As before, I investigated socio-demographics, psychosocial stressors, illness perceptions, baseline psychopathology and baseline health status. In order to divide the patients into those with and without IFN-α-induced depression, I used the MINI diagnostic interview to confirm a diagnosis of MDD. A total of 19 patients (40%) developed IFN-α-induced depression at some point during the course of the 24-week therapy, while 29 patients (60%) did not develop IFN-α-induced depression. The cumulative percentage of patients that developed IFN-α-induced depression at each week is presented in Figure 3.33.

3.1.4.1 Socio-demographic characteristics of patients with and without IFN-α-induced depression

Patients who developed IFN-α-induced depression had a higher prevalence of unemployment as well as a higher prevalence of a previous history of MDD, when compared with patients who did not develop IFN-α-induced depression ($p=0.010$ and $p=0.044$ respectively). There were no significant differences between the two groups for age, gender, education level, relationship status, family history of psychiatric illness or for history of substance use (opioids). The two groups were also not significantly different in their HCV genotype, their viral load or their fibroscan scores ($p=0.6$, $p=0.8$ and $p=0.8$, respectively). These data are presented in Table 3.11.

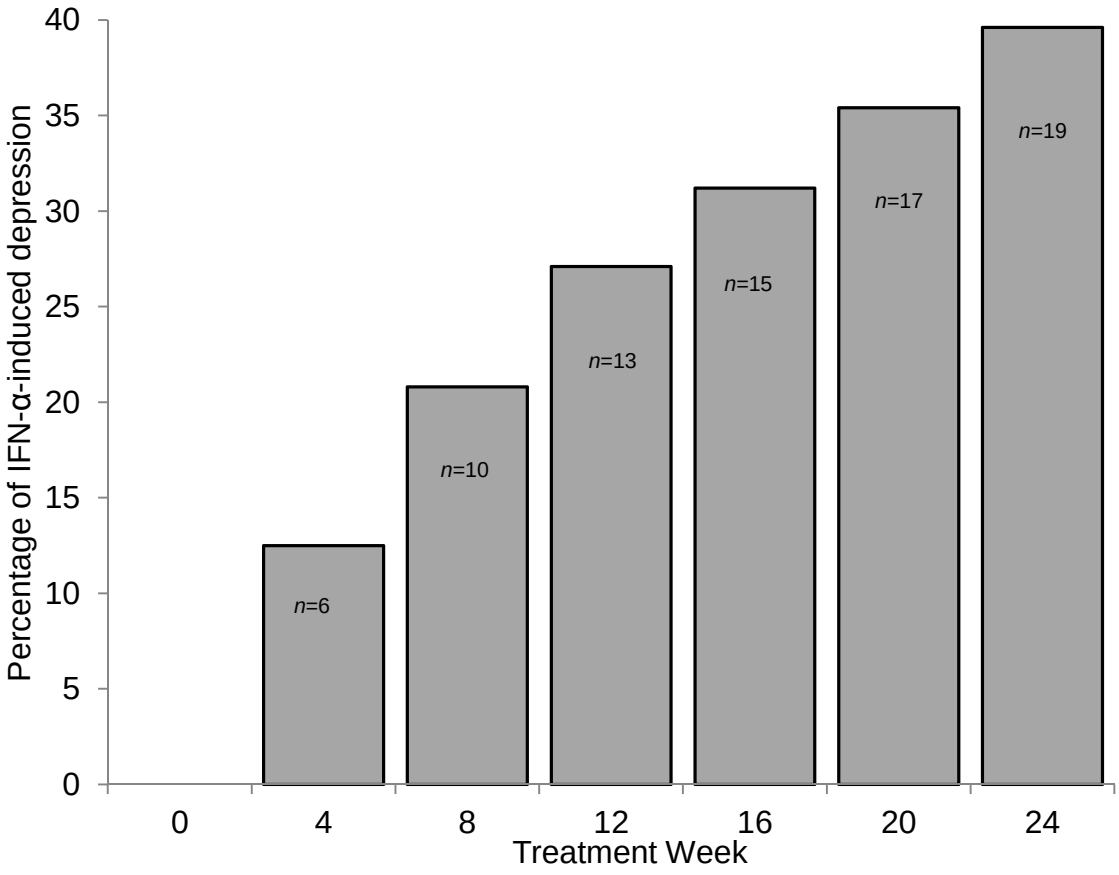


Figure 3.33 The cumulative percentage of patients who developed IFN-α-induced depression

The cumulative percentage of patients meeting a MINI diagnosis of current major depressive episode at each IFN-α treatment week.

Table 3.11 Socio-demographic characteristics of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 29**	
Age (years)			
Mean±SEM	42.1±2.7	44.0±2.1	*t*=0.6, df=46, p=0.6
Gender			
Males	14 (74%)	22 (76%)	x^2=0.03, p=0.6
Ethnicity			
White British	9 (47%)	14 (48%)	x^2=<0.01, p=0.6
Education Level			
University Degree	6 (32%)	9 (31%)	x^2=<0.01, p=0.6
Employment			
Unemployed	11 (58%)	6 (21%)	x^2=7.0, **p=0.010**
Relationship Status			
Single	8 (42%)	16 (55%)	x^2=0.8, p=0.3
History of MDD	10 (53%)	7 (24%)	x^2=4.1, **p=0.044**
Family History	5 (26%)	8 (28%)	x^2=<0.01, p=0.6
Substance Use	6 (32%)	11 (38%)	x^2=0.2, p=0.5
HCV Genotype			
1	5 (26%)	6 (21%)	
2	3 (16%)	2 (7%)	x^2=1.9, p=0.6
3	11 (58%)	20 (69.%)	
4	0 (0%)	1 (3%)	
HCV Viral Load (million)			
Mean±SEM	2.0±0.7	2.2±0.6	*t*=0.9, df=44, p=0.8
Fibroscan Score (kpa)			
Mean±SEM	8.6±1.6	9.1±1.6	*t*=0.2, df=36, p=0.8

3.1.4.2 The psychosocial stress characteristics of patients with and without IFN-α-induced depression

Patients with IFN-α-induced depression had a higher prevalence of experiencing at least one stressful life event when compared with patients without IFN-α-induced depression, however this was not statistically significant (58% vs. 38%, p=0.1). The two groups were also not significantly different in their reports of any of the four forms of childhood traumatic events. These data are presented in Table 3.12.

3.1.4.3 The illness perceptions scores of patients with and without IFN-α-induced depression

The two groups were not significantly different in their scores of any of the seven illness perceptions dimensions. However, patients with IFN-α-induced depression had slightly higher scores on the timeline as well as the consequences dimensions suggesting that they perceived their illness to be more chronic in nature and with more negative consequences when compared with patients without IFN-α-induced depression (16.1±1.2 vs. 13.8±1.2 and 18.7±1.2 vs. 17.9±1.2, respectively). Furthermore, patients with IFN-α-induced depression had slightly lower scores on the personal control dimension when compared with patients without IFN-α-induced depression (21.8±0.7 vs. 23.6±0.8), thus perceiving themselves to be less able to control their symptoms and their illness. These data are presented in Table 3.13.

Table 3.12 Psychosocial stress characteristics of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 29**	
Brief Life Events			
Yes	11 (58%)	11 (38%)	x^2=1.8, p=0.1
Parental Separation			
Yes	3 (16%)	9 (31%)	x^2=2.2, p=0.1
Parental Loss			
Yes	1 (5%)	4 (14%)	x^2=1.2, p=0.3
Physical Abuse			
Yes	2 (11%)	5 (17%)	x^2=0.7, p=0.3
Sexual Abuse			
Yes	3 (16%)	5 (17%)	x^2=0.1, p=0.5
Any Trauma			
Yes	7 (37%)	12 (41%)	x^2=0.6, p=0.3

Table 3.13 Illness perceptions scores of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 29**	
Timeline *(acute/chronic)*	16.1±1.2	13.8±1.2	*t*=-1.2, df=44, p=0.2
Consequences	18.7±1.2	17.9±1.2	*t*=-0.4, df=44, p=0.6
Timeline *(cyclical)*	9.8±0.8	8.8±0.8	*t*=-0.8, df=43, p=0.4
Personal Control	21.8±0.7	23.6±0.8	*t*=1.6, df=43, p=0.1
Treatment Control	22.4±1.6	20.9±0.6	*t*=-1.0, df=43, p=0.3
Illness Coherence	18.6±0.8	18.8±0.8	*t*=-0.3, df=44, p=0.8
Emotional Representations	18.3±1.4	16.9±1.3	*t*=-0.7, df=43, p=0.5

3.1.4.4 The baseline psychopathology of patients with and without IFN-α-induced depression

Although not statistically significant, patients who developed IFN-α-induced depression had higher baseline depression scores (p=0.1). Moreover, baseline scores of these patients were already above the cut-off considered to be within normal range (<12) and were within the range considered to be mild depression (13-23). Patients who developed IFN-α-induced depression also had slightly higher baseline fatigue scores however, both groups were within the normal range (≤18), and this difference was not significant (p=0.1). Similarly, patients who developed IFN-α-induced depression had slightly higher baseline stress scores, however this difference was also not significant (p=0.3). Baseline anxiety scores were comparable between the two groups (p=0.7). These data are presented in Table 3.14.

3.1.4.5 The baseline health status of patients with and without IFN-α-induced depression

On the 8 dimensions of the SF-36 (where lower scores indicate worse functioning), there were significant differences at baseline between the two groups on 3 of the 8 dimensions; vitality, bodily pain and general health. Patients who later developed IFN-α-induced depression already had lower vitality scores, more bodily pain and worse general health when compared to patients who did not develop IFN-α-induced depression (p=0.036, p=0.026 and p=0.024, respectively). The two groups were not significantly different in their scores for the physical functioning, physical or emotional role limitation, mental health or social functioning dimensions of the SF-36 (p=0.1, p=0.8, p=0.2, p=0.2 and p=0.4, respectively). These data are presented in Table 3.15.

Table 3.14 Baseline psychopathology of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 29**	
IDS	15.6±3.1	9.4±1.6	*t*=-1.9, df=45, p=0.1
CFQ	13.7±2.0	11.7±0.8	*t*=-1.5, df=44, p=0.1
PSS	13.4±1.4	10.9±1.5	*t*=-1.1, df=45, p=0.3
HADS-A	5.1±1.0	4.7±0.7	*t*=-0.4, df=45, p=0.7

Table 3.15 Baseline health status of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 29**	
SF-36 *Physical functioning*	83.2±3.6	89.8±2.7	*t*=1.5, df=46, p=0.1
SF-36 *Physical role limitation*	72.4±9.9	75.9±7.2	*t*=0.3, df=46, p=0.8
SF-36 *Emotional role limitation*	70.2±10.2	85.1±5.6	*t*=1.4, df=46, p=0.2
SF-36 *Vitality*	50.5±4.6	64.7±4.4	*t*=2.2, df=46, **p=0.036**
SF-36 *Mental health*	71.4±3.7	77.9±3.4	*t*=1.3, df=46, p=0.2
SF-36 *Social functioning*	75.7±7.1	82.3±5.1	*t*=0.8, df=46, p=0.4
SF-36 *Bodily pain*	68.0±6.2	83.5±3.6	*t*=2.3, df=46, **p=0.026**
SF-36 *General health*	49.0±5.4	64.1±3.9	*t*=2.3, df=46, **p=0.024**

3.1.5 Psychopathological changes in patients with and without IFN-α-induced depression

For this section, I have again divided the patients in to those with and without IFN-α-induced depression and present the effect of depression status on each variable using a random intercept regression model with maximum likelihood effects. Depression scores were higher in patients with IFN-α-induced depression at baseline and throughout the treatment period, with a rapid increase in scores in both groups between baseline and treatment week 4. However, depression scores of patients with IFN-α-induced depression continued to increase until treatment week 12. Of note, depression scores of patients without IFN-α-induced depression remained within the range considered to be mild depression (13-23) whereas depression scores of patients with IFN-α-induced depression reached the range considered as moderate depression (24-36). As expected, there was a significant association between depression status and depression scores (Coefficient=15.6, $p<0.001$). This remained significant after adjusting for baseline depression scores ($p<0.001$), indicating that depression status is associated with increased depression scores during treatment irrespective of baseline depression levels. These data are presented in Figure 3.34 and Figure 3.35.

Fatigue scores increased in both groups but remained slightly higher in patients with IFN-α-induced depression. However, there was no significant association between depression status and fatigue scores ($p=0.1$).

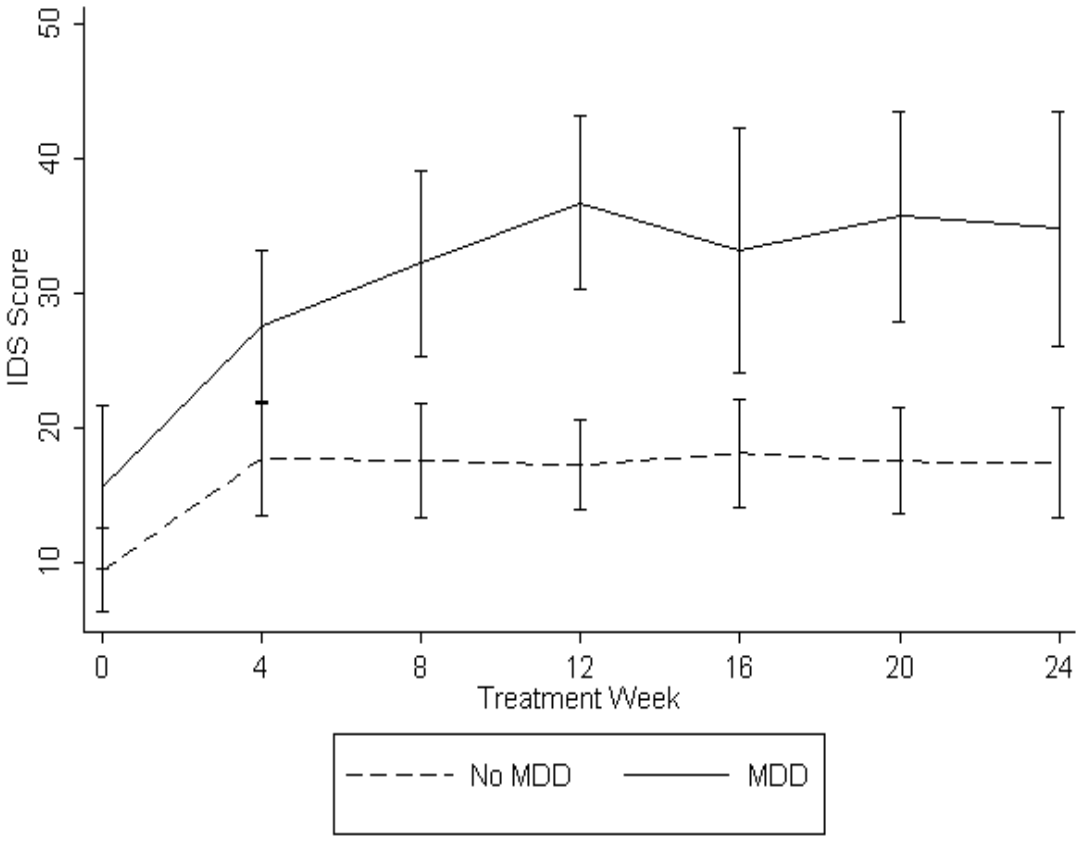


Figure 3.34 Changes in mean depression scores of patients with and without IFN-α-induced depression

Changes in mean scores on the Inventory of Depressive Symptomatology (IDS) across the 24 weeks of treatment in patients with (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

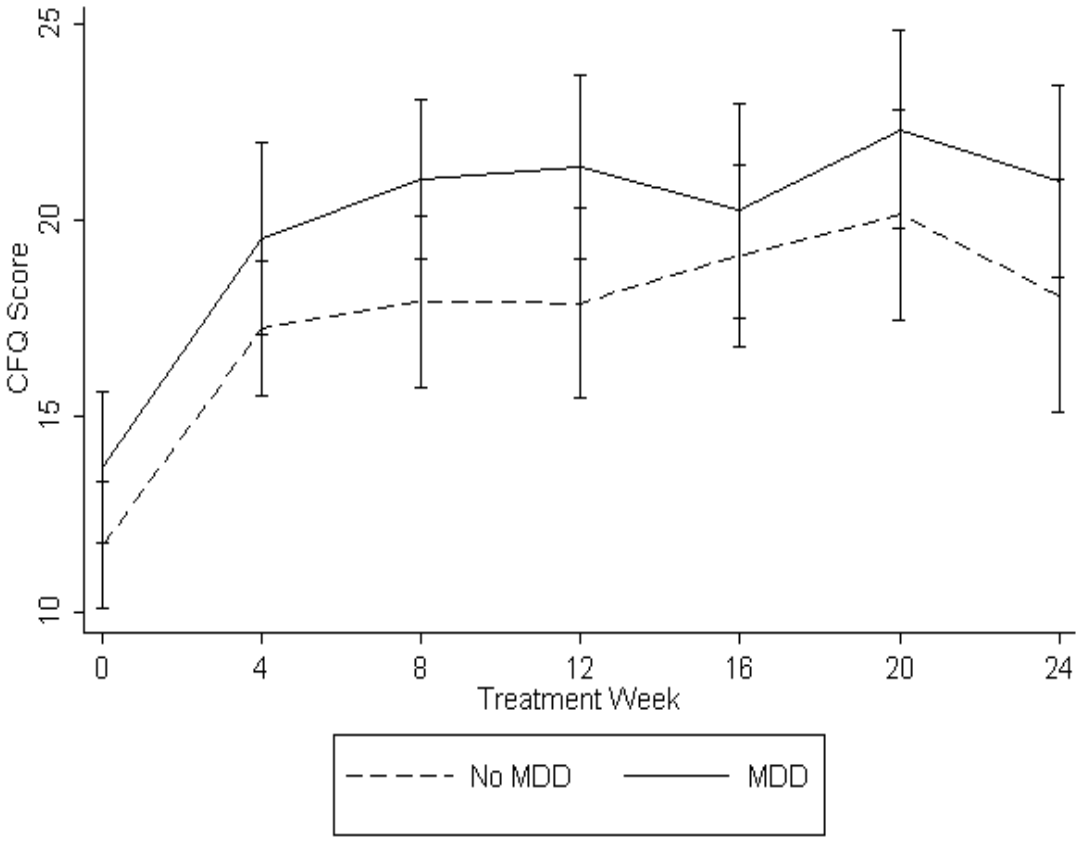


Figure 3.35 Changes in mean fatigue scores of patients with and without IFN-α-induced depression

Changes in mean scores on the Chalder Fatigue Questionnaire (CFQ) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

Changes in stress and anxiety scores during IFN-α treatment in patients with and without IFN-α-induced depression are presented in Figure 3.36 and Figure 3.37. Stress scores gradually increased between baseline and treatment week 12 and remained elevated until the end of treatment in patients with IFN-α-induced depression. Interestingly, in patients without IFN-α-induced depression there were very little changes, and stress scores remained relatively stable throughout the treatment period. There was a significant association between depression status and stress scores (Coefficient=9.1, $p<0.001$) which remained significant after adjusting for baseline stress scores ($p<0.001$) indicating that depression status is associated with increased stress scores during treatment irrespective of baseline stress levels.

Finally, similar to stress scores, anxiety scores gradually increased between baseline and treatment week 12 and remained elevated until the end of treatment in patients with IFN-α-induced depression. In patients without IFN-α-induced depression, anxiety scores decreased slightly but remained relatively stable throughout the treatment period. Of note, anxiety scores of patients without IFN-α-induced depression remained within the range considered to be normal (0-7) whereas anxiety scores of patients with IFN-α-induced depression reached the range considered to be mild anxiety (8-10). There was a significant association between depression status and anxiety scores (Coefficient=4.2, $p<0.001$) which remained significant after adjusting for baseline anxiety scores ($p<0.001$) indicating that depression status is associated with increased anxiety scores during treatment irrespective of baseline anxiety levels.

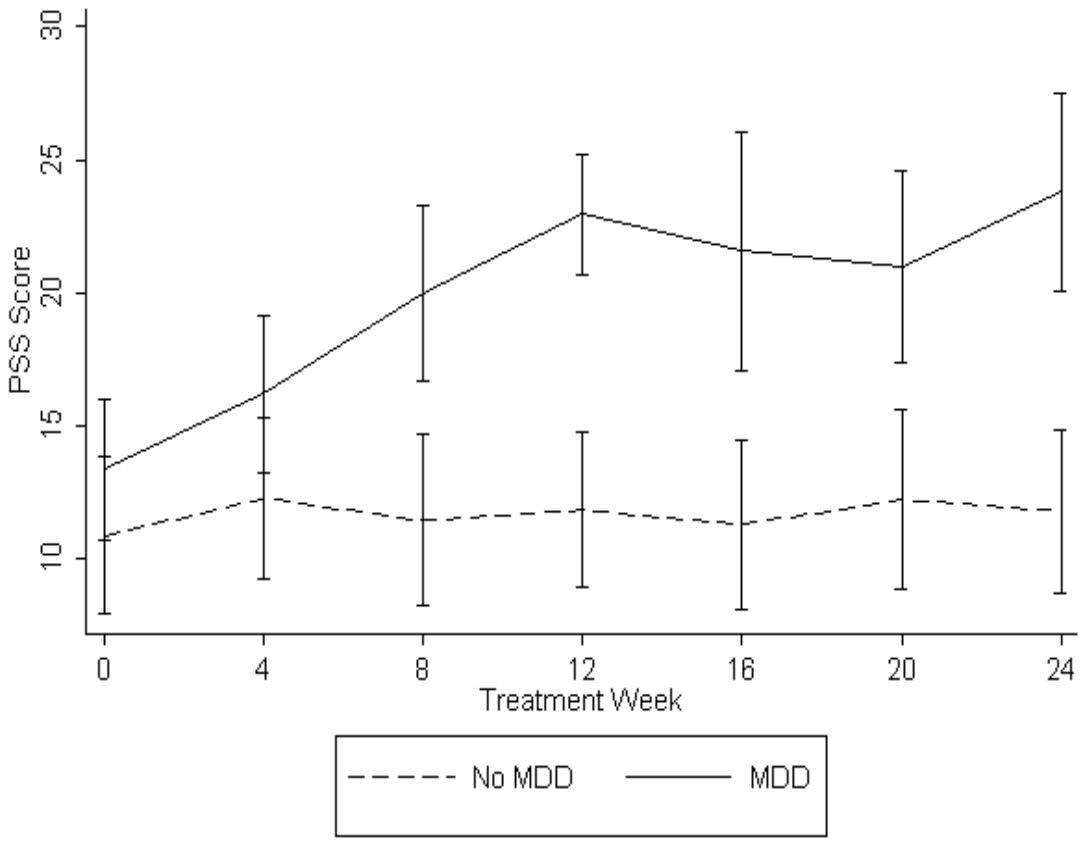


Figure 3.36 Changes in mean stress scores of patients with and without IFN-α-induced depression

Changes in mean scores on the Perceived Stress Scale (PSS) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

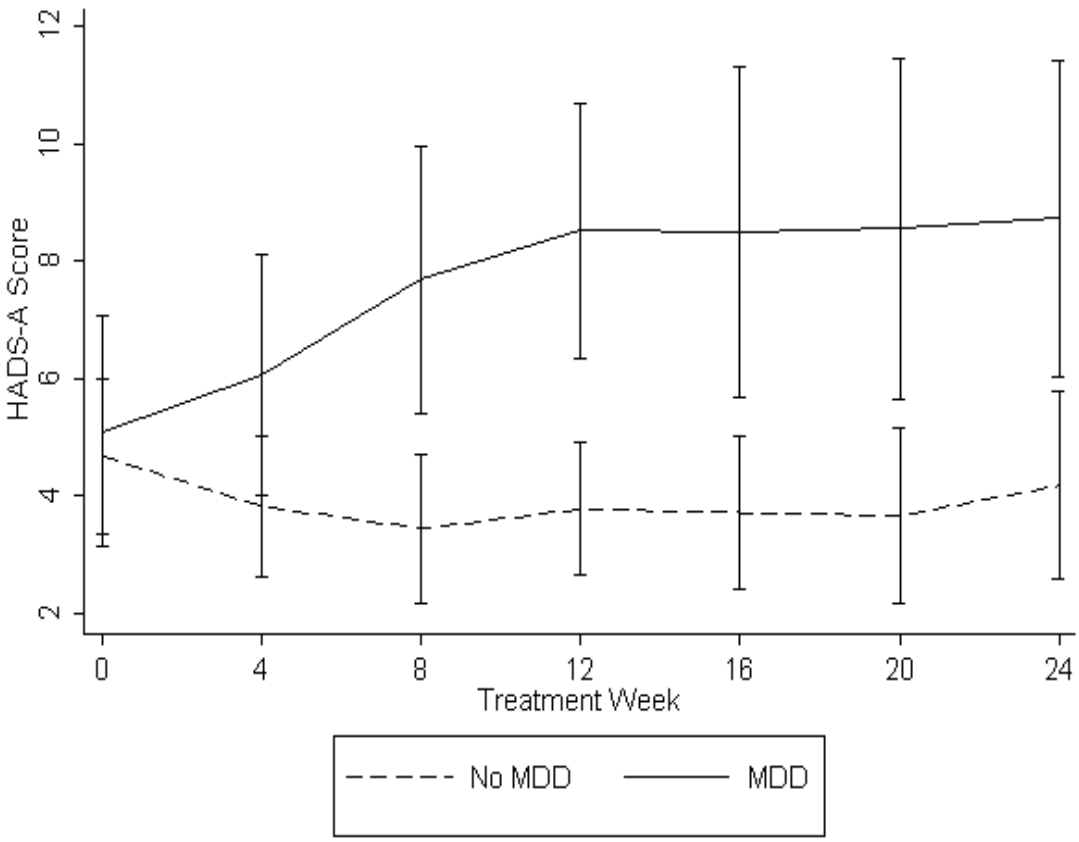


Figure 3.37 Changes in mean anxiety scores of patients with and without IFN-α-induced depression

Changes in mean scores on the anxiety sub-scale of the Hospital Anxiety and Depression Scale (HADS-A) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

3.1.6 Health status changes in patients with and without IFN-α-induced depression

I also investigated the changes in health status (SF-36 scores) of patients with and without IFN-α-induced depression across the treatment course. These data are presented in Figure 3.38-Figure 3.45. As mentioned previously, for all 8 dimensions, higher scores represent better functioning with a score of 100 indicating optimal well-being. There was a significant association between depression status and scores on the physical functioning, physical role limitation and social functioning dimensions (Coefficient=-16.0, p=0.010; Coefficient=-25.3, p=0.008 and Coefficient=-19.3, p=0.007, respectively). These effects all remained significant after adjusting for the baseline scores for these dimensions (p=0.037, p=0.004 and p=0.011, respectively). There was a particularly strong association between depression status and scores on the emotional role limitation and mental health domains (Coefficient=-37.9, p<0.001 and Coefficient=-23.2, p<0.001) with both remaining highly significant even after adjusting for the respective baseline scores (p<0.001 and p<0.001). These data indicate that depression status is associated with decreased well-being in these health status dimensions irrespective of baseline levels of well-being. Lastly, there was a significant association between depression status and scores on the vitality, bodily pain and general health dimensions (Coefficient=-14.9, p=0.029; Coefficient=-17.8, p=0.006 and Coefficient=-15.8, p=0.008, respectively). However, after adjusting for baseline scores in these three dimensions, these effects were no longer significant (p=0.4, p=0.1 and p=0.1, respectively) indicating that baseline vitality, bodily pain and general health status scores drive these effects.

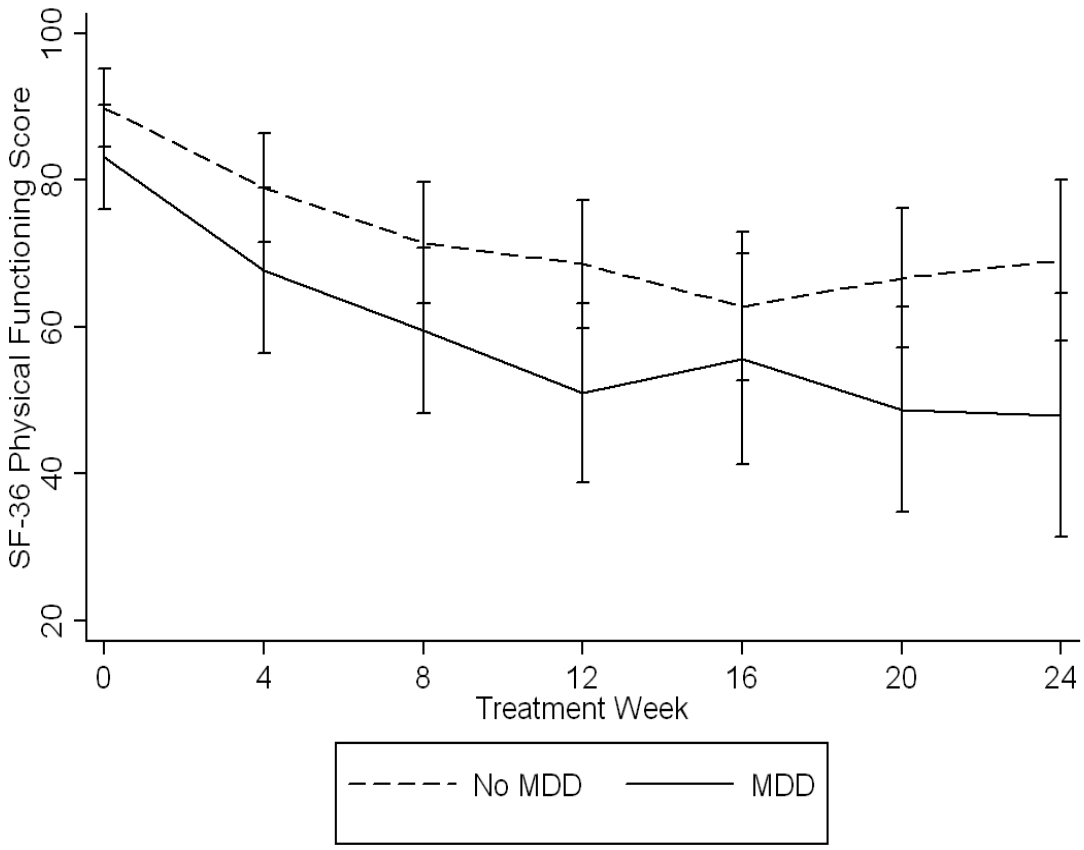


Figure 3.38 Changes in mean physical functioning scores of patients with and without IFN-α-induced depression

Changes in mean scores on the physical functioning dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

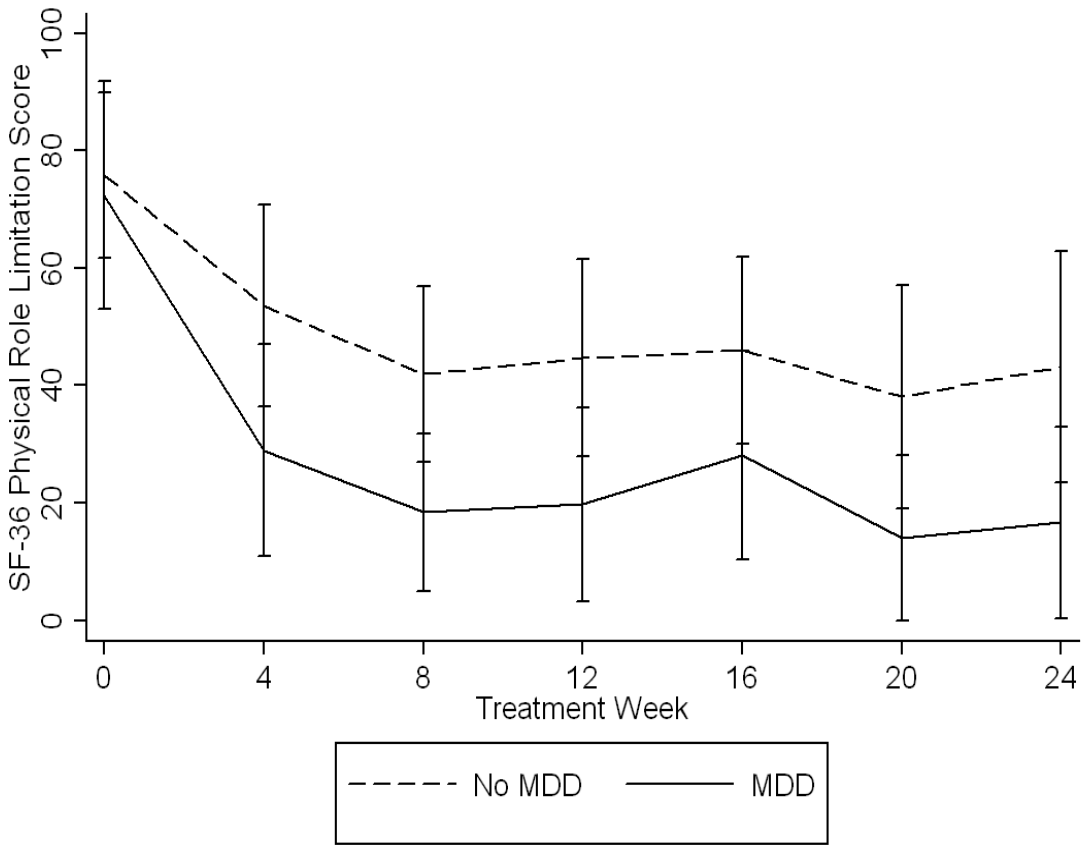


Figure 3.39 Changes in mean physical role limitation scores of patients with and without IFN-α-induced depression

Changes in mean scores on the physical role limitation dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

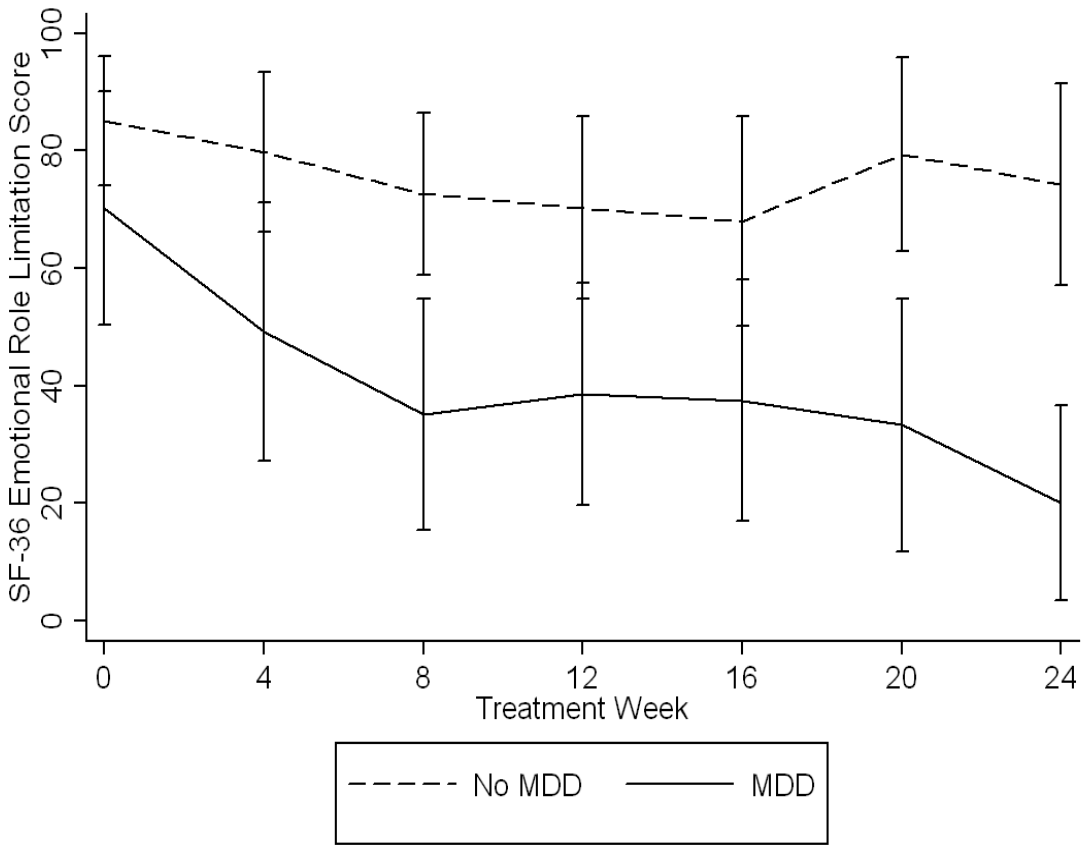


Figure 3.40 Changes in mean emotional role limitation scores of patients with and without IFN-α-induced depression

Changes in mean scores on the emotional role limitation dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

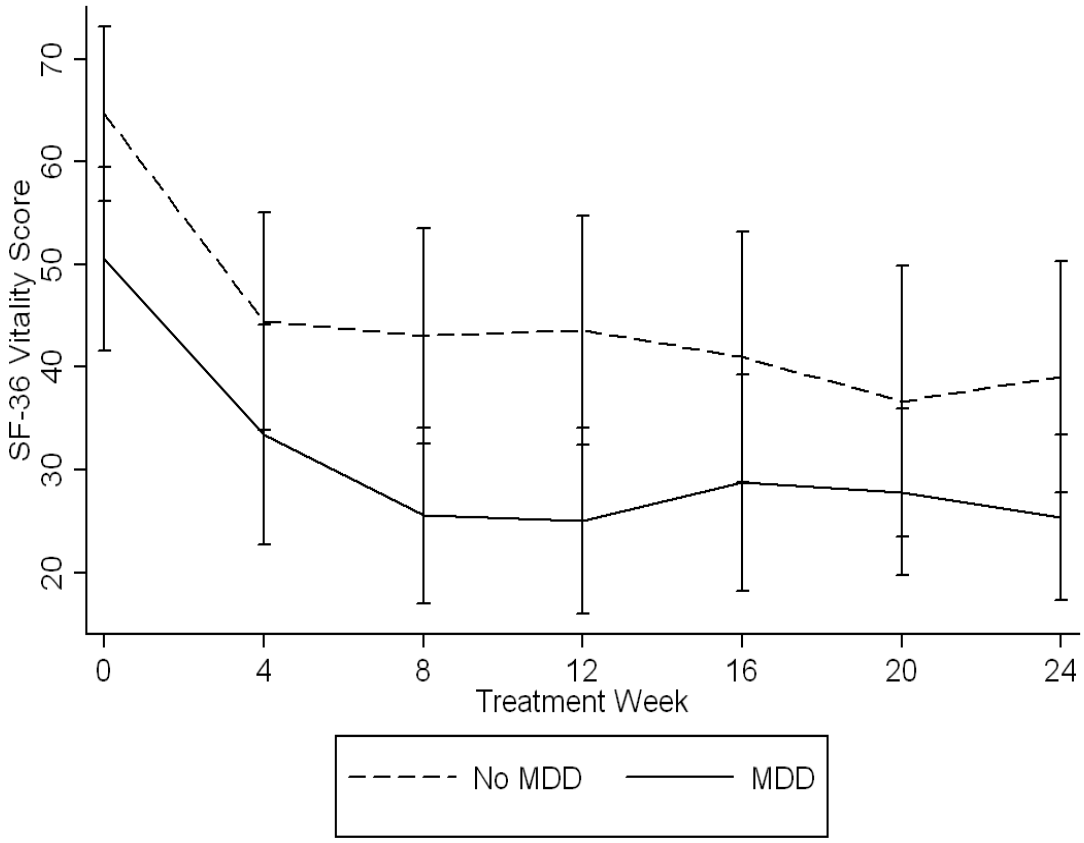


Figure 3.41 Changes in mean vitality scores of patients with and without IFN-α-induced depression

Changes in mean scores on the vitality dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

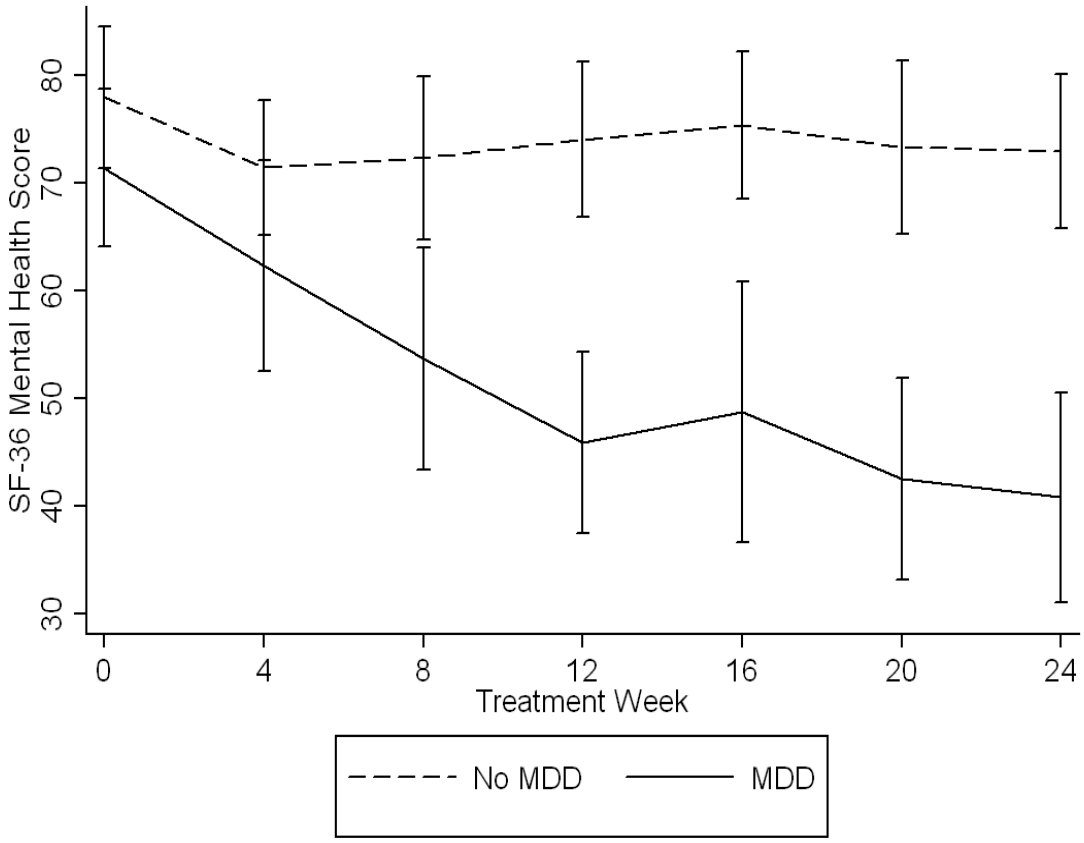


Figure 3.42 Changes in mean mental health scores of patients with and without IFN-α-induced depression

Changes in mean scores on the mental health dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

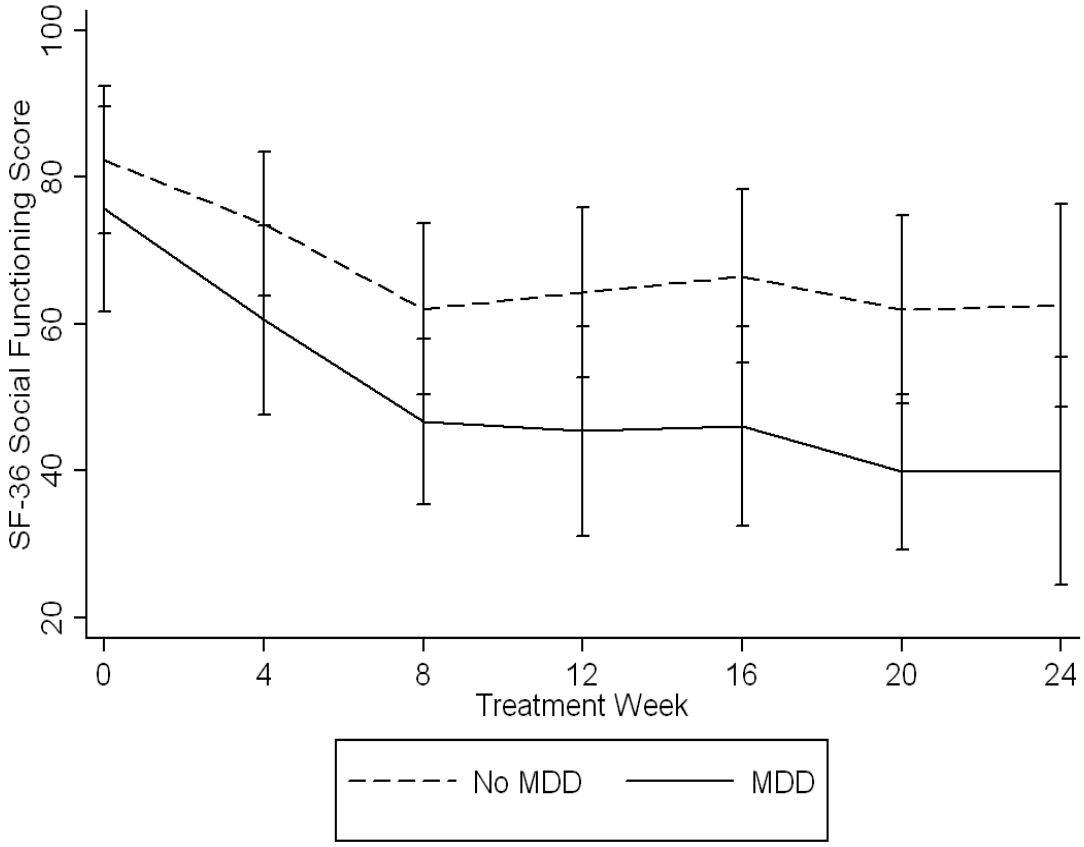


Figure 3.43 Changes in mean social functioning scores of patients with and without IFN-α-induced depression

Changes in mean scores on the social functioning dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

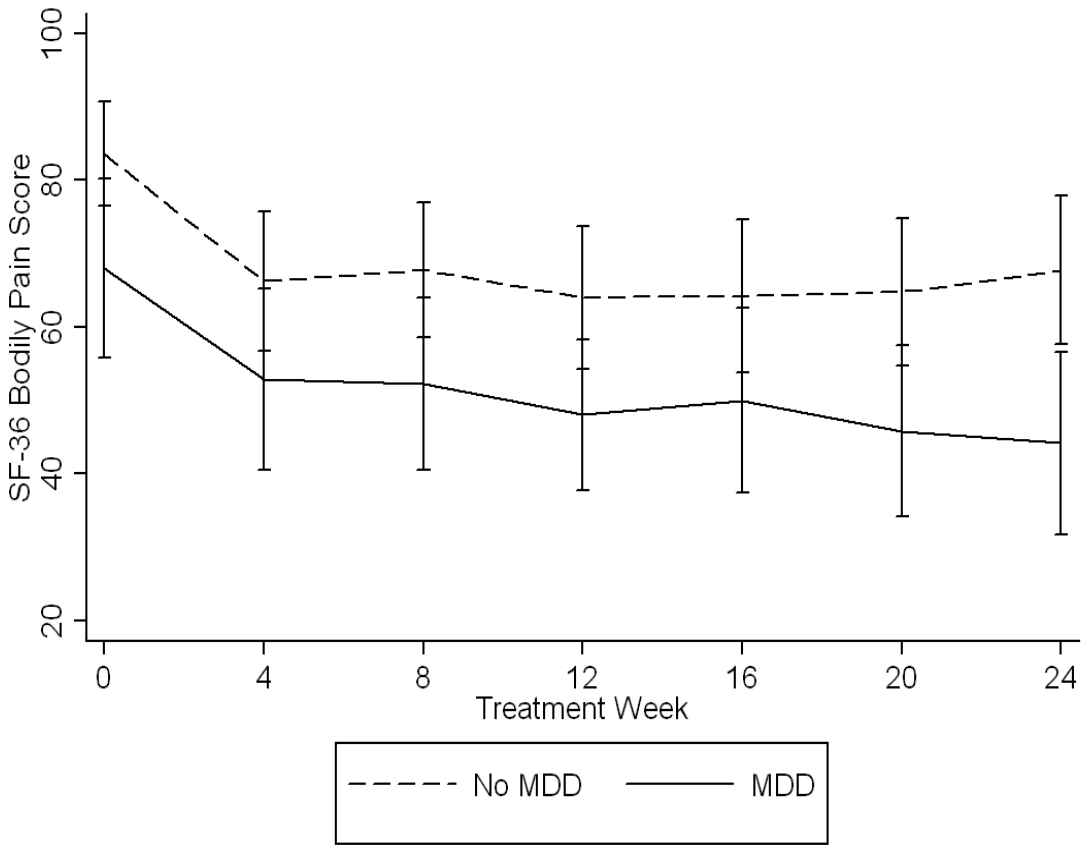


Figure 3.44 Changes in mean bodily pain scores of patients with and without IFN-α-induced depression

Changes in mean scores on the bodily pain dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

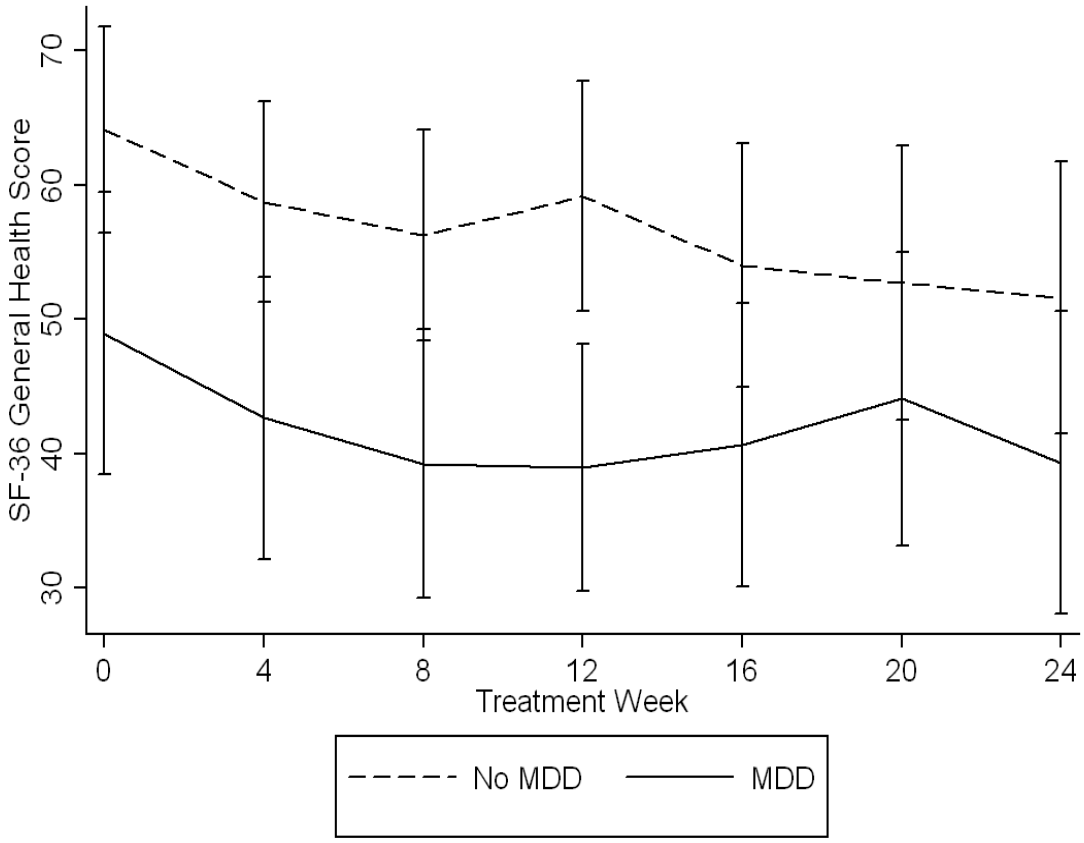


Figure 3.45 Changes in mean general health scores of patients with and without IFN-α-induced depression

Changes in mean scores on the general health dimension of the Medical Outcomes Study Short-Form 36 (SF-36) across the 24 weeks of treatment in patients with IFN-α-induced depression (*n* ranging from 15-19) and patients without IFN-α-induced depression (*n* ranging from 21-29).

3.1.7 Biological changes in patients with and without IFN-α-induced depression

In order to investigate biological differences, I have again divided the patients in to those with and without IFN-α-induced depression. I present the effect of depression status on each biological variable using a random intercept regression model with maximum likelihood effects.

3.1.7.1 Cortisol

Differences in baseline cortisol output in patients with and without IFN-α-induced depression are shown in Table 3.16. These analyses were conducted on a small number of patients (4 depressed and 15 non-depressed, since overall the cortisol samples were only available in a sub-sample). There were no significant cortisol differences between patients with and without IFN-α-induced depression at baseline.

Changes in the cortisol awakening response from baseline to treatment week 24 in patients with and without IFN-α-induced depression are presented in Figure 3.46 and Figure 3.47. At baseline, although not significant, patients who later develop IFN-α-induced depression appear to have an increased cortisol awakening response compared to patients without IFN-α-induced depression (p=0.1). However, this difference is completely diminished at treatment week 24 (Coefficient=0.4, p=0.9). I then investigated changes in the AUCi of the cortisol awakening response from baseline to treatment week 24, in patients with and without IFN-α-induced depression, as shown in Figure 3.48. There was a decrease in the AUCi of the cortisol awakening response from baseline to treatment week 24 in patients with IFN-α-induced depression, and no change in patients without IFN-α-induced depression. However, there was no significant

effect of depression status on the change in the AUCi of the cortisol awakening response (p=0.3). Independent samples t-tests also confirmed there was no significant difference in the AUCi of the cortisol awakening response between the two patient groups at baseline (*t*=-1.4, df=15, p=0.2) or at treatment week 24 (*t*=-0.1, df=9, p=0.9) however this may be due to the small sample size.

Table 3.16 Baseline cortisol levels in patients with and without IFN-α-induced depression

	MDD ***n* = 4**	**No MDD** ***n* = 15**	
Awakening AUCi	116.7±3.0	-29.9±46.6	*t*=-1.4, df=15, p=0.2
Delta 15	1.0±3.0	-0.2±0.8	*t*=-0.6, df=15, p=0.6
Delta 30	2.4±3.1	-0.3±0.9	*t*=-1.2, df=15, p=0.3
Delta 60	3.2±1.6	-1.6±1.2	*t*=-2.0, df=16, p=0.1
Day AUC	2071.9 ± 681.4	27727.2 ± 376.6	*t*=0.9, df=17, p=0.4
Noon	3.7±1.4	3.0±0.5	*t*=-0.6, df=17, p=0.6
8PM	1.2±0.6	2.0±0.5	*t*=0.8, df=17, p=0.4

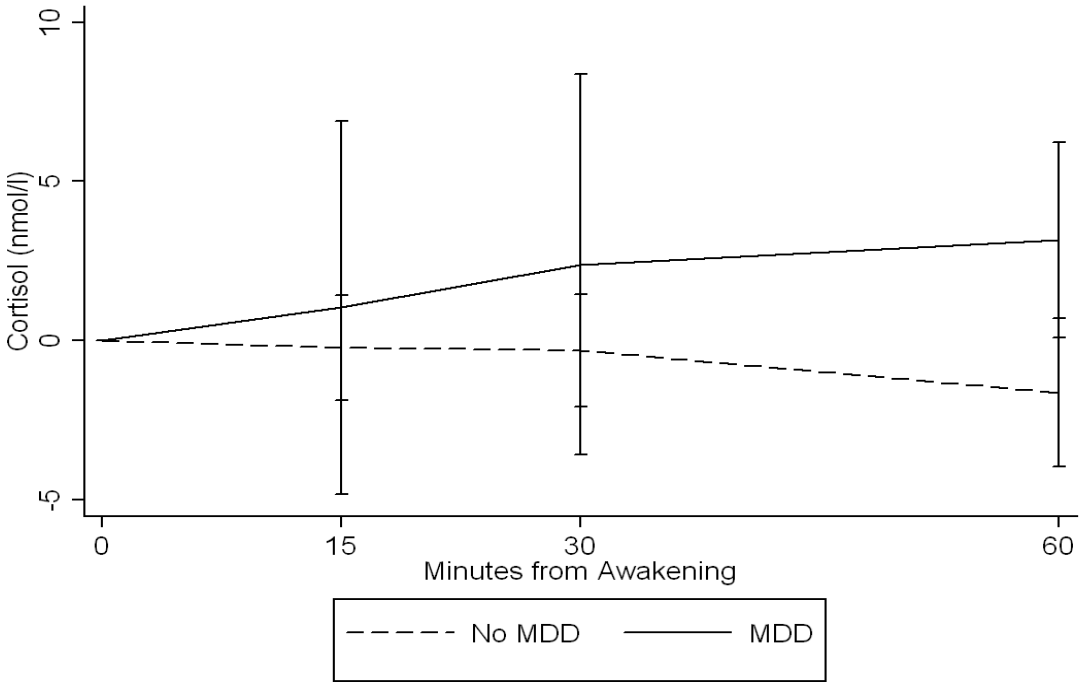


Figure 3.46 The cortisol awakening response at baseline of patients with (*n*=4) and without (*n*=15) IFN-α-induced depression

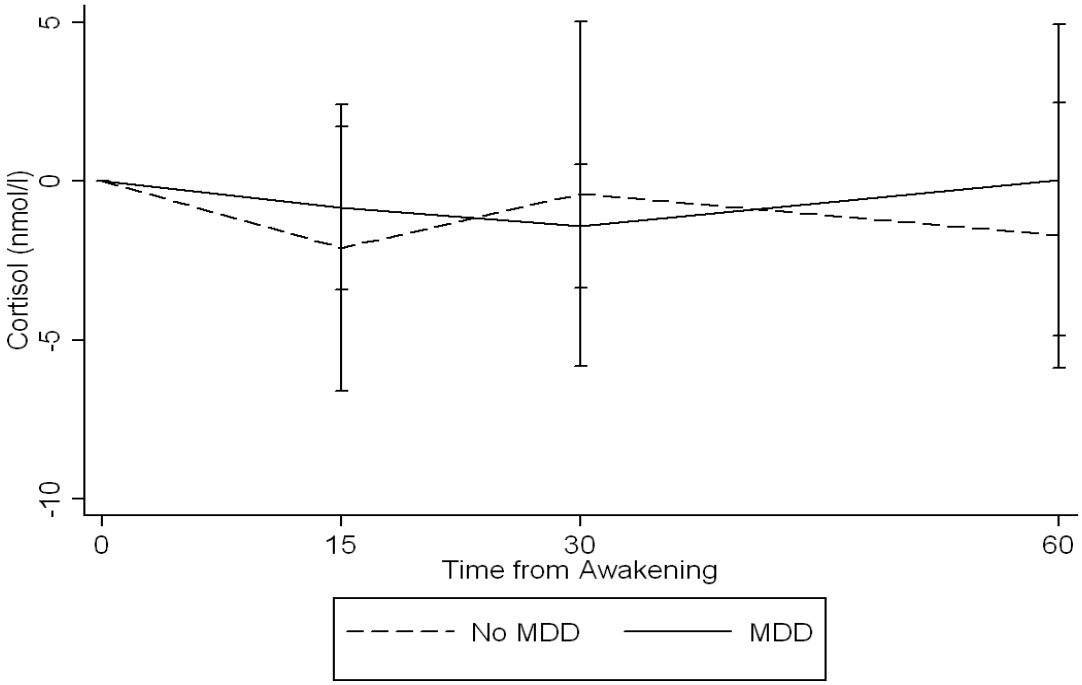


Figure 3.47 The cortisol awakening response at treatment week 24 of patients with (*n*=2) and without (*n*=9) IFN-α-induced depression

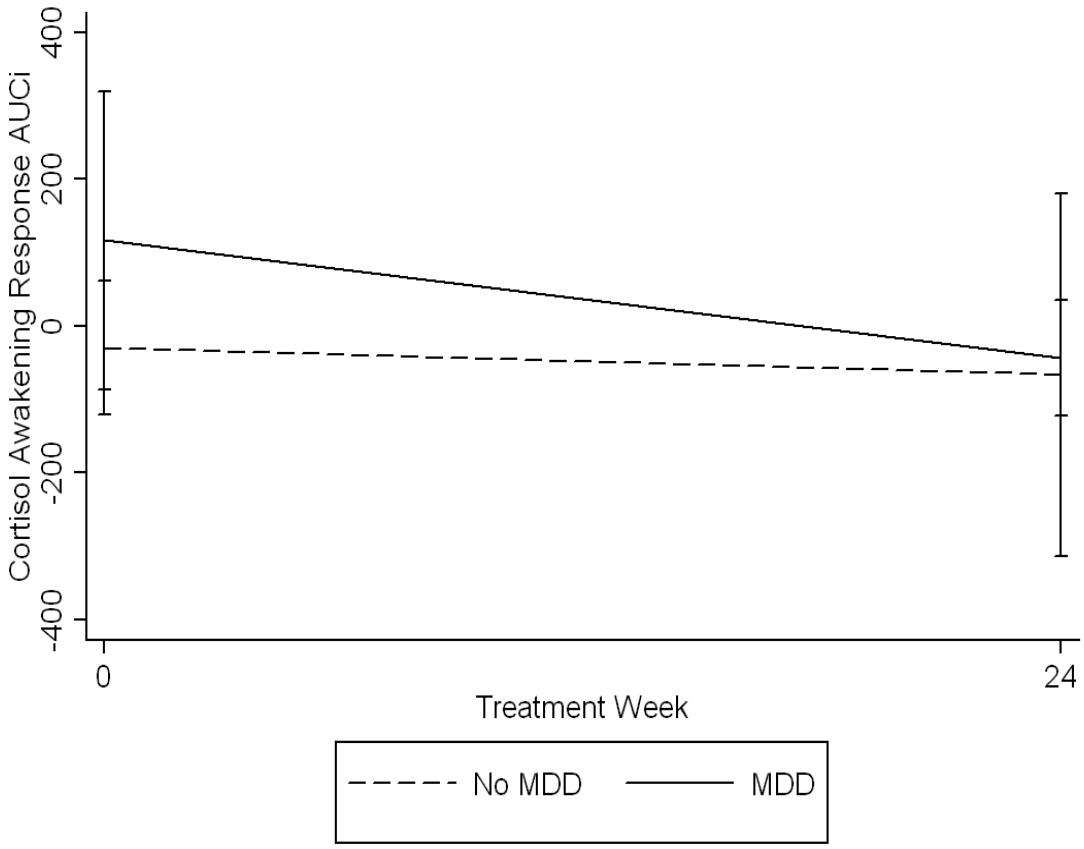


Figure 3.48 Changes in the area under the curve of the increase (AUCi) of the cortisol awakening response from baseline to treatment week 24 in patients with (*n*=2) and without (*n*=9) IFN-α-induced depression

I also investigated the changes in cortisol levels during the day from baseline to treatment week 24 in patients with and without IFN-α-induced depression. Again, I firstly looked at the changes in the raw values of cortisol at 0 minutes (awakening), noon and 8pm from baseline to treatment week 24 of IFN-α treatment as shown in Figure 3.49 and Figure 3.50. At baseline, patients with IFN-α-induced depression have a flattening of the cortisol response that is, a smaller difference between the morning peak and the evening trough. However, this effect is diminished at treatment week 24 at which point the two groups are very similar. However, there was no significant effect of depression status on cortisol levels during the day at either baseline or treatment week 24 ($p=0.4$ and $p=0.9$, respectively).

As shown in Figure 3.51, I then also investigated the change in the AUC of cortisol during the day from baseline to treatment week 24 in the two groups. There was an increase in the AUC of cortisol during the day from baseline to treatment week 24 in patients with IFN-α-induced depression whereas patients without IFN-α-induced depression appear to have no change. However, there was no significant effect of depression status on the changes in the AUC of cortisol during the day ($p=0.6$) which again may be due the small sample size.

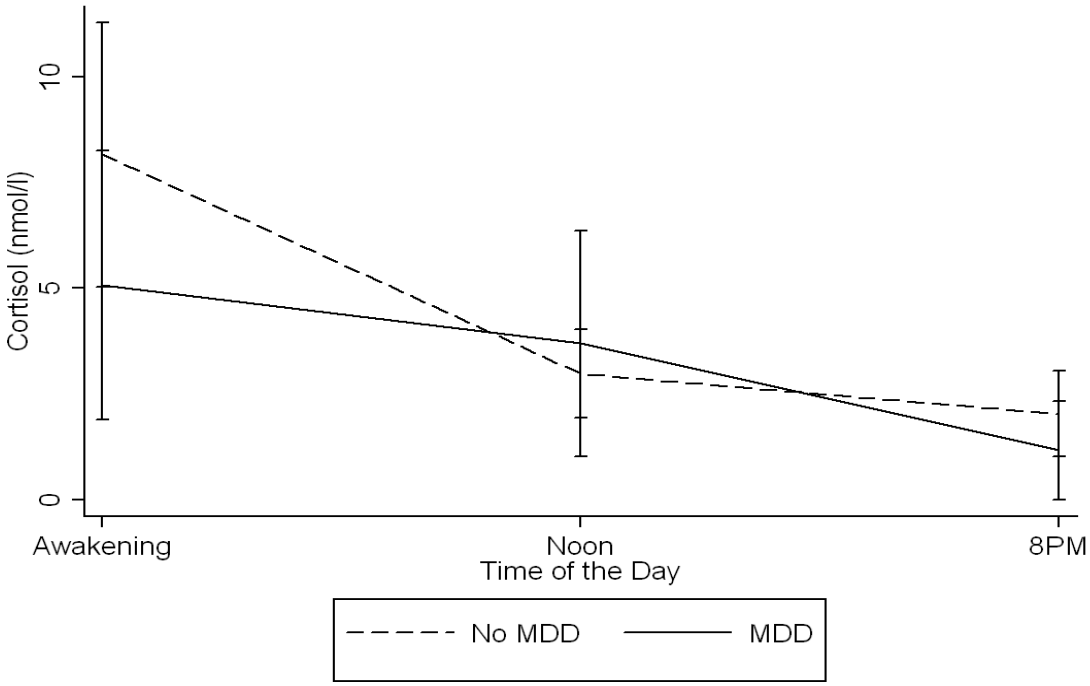


Figure 3.49 Cortisol levels during the day at baseline of patients with (*n*=4) and without (*n*=15) IFN-α-induced depression

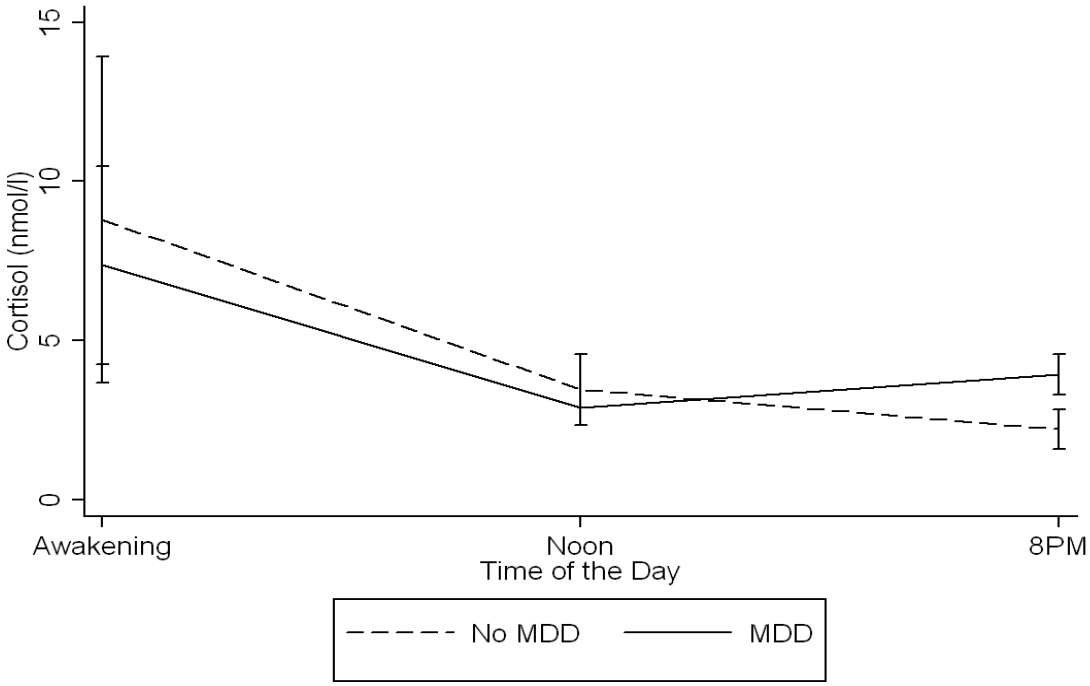


Figure 3.50 Cortisol levels during the day at treatment week 24 of patients with (*n*=2) and without (*n*=9) IFN-α-induced depression

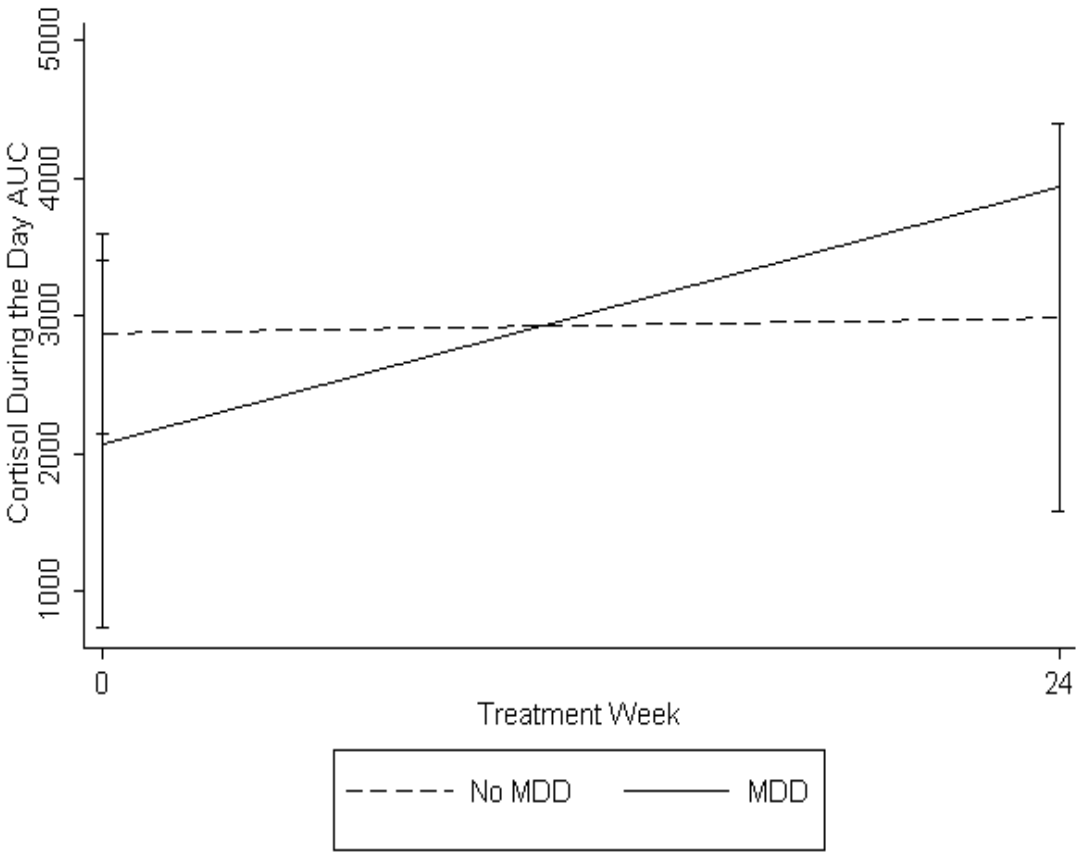


Figure 3.51 Changes in the area under the curve (AUC) of cortisol during the day from baseline to treatment week 24 of patients with (*n*=2) and without (*n*=9) IFN-α-induced depression

3.1.7.2 Kynurenine and Tryptophan pathway

The differences in baseline levels of kynurenine and tryptophan pathway metabolites in patients with and without IFN-α-induced depression are shown in Table 3.17. There were no significant differences between the two groups for any of the kynurenine and tryptophan pathway metabolites. Changes in the levels of kynurenine and tryptophan pathway metabolites in patients with and without IFN-α-induced depression during the treatment period are shown in Figure 3.52-Figure 3.56. There was no significant effect of depression status on tryptophan or kynurenic acid levels ($p=0.8$ and $p=0.7$, respectively). Kynurenine and three-hydroxykynurenine levels increased in both groups with no significant effect of depression status ($p=0.8$ and $p=0.9$, respectively). Finally, there was no significant effect of depression status on the ratio of kynurenine to tryptophan ($p=0.6$). Independent samples t-tests also confirmed that there were no significant differences between patients with and without IFN-α-induced depression for any of the kynurenine and tryptophan pathway metabolites at treatment week 8 or at treatment week 24, indicating that IFN-α-induced changes in kynurenine and tryptophan metabolites are the same in all patients.

Table 3.17 Baseline kynurenine and tryptophan pathway metabolites levels in patients with and without IFN-α-induced depression

	MDD ***n* = 17**	**No MDD** ***n* = 21**	
Tryptophan ug/l	13.1±2.7	13.2±0.4	*t*=0.03, df=36, p=1.0
Kynurenine ng/l	421.8±21.8	439.4±19.1	*t*=0.6, df=36, p=0.6
3-HK ng/l	10.4±1.1	10.2±0.5	*t*=-0.1, df=36, p=0.9
Kynurenic acid ng/l	6.4±0.5	6.3±0.5	*t*=-0.2, df=36, p=0.8
Kynurenine/ Tryptophan ratio	33.2±2.1	33.9±1.7	*t*=0.3, df=36, p=0.8

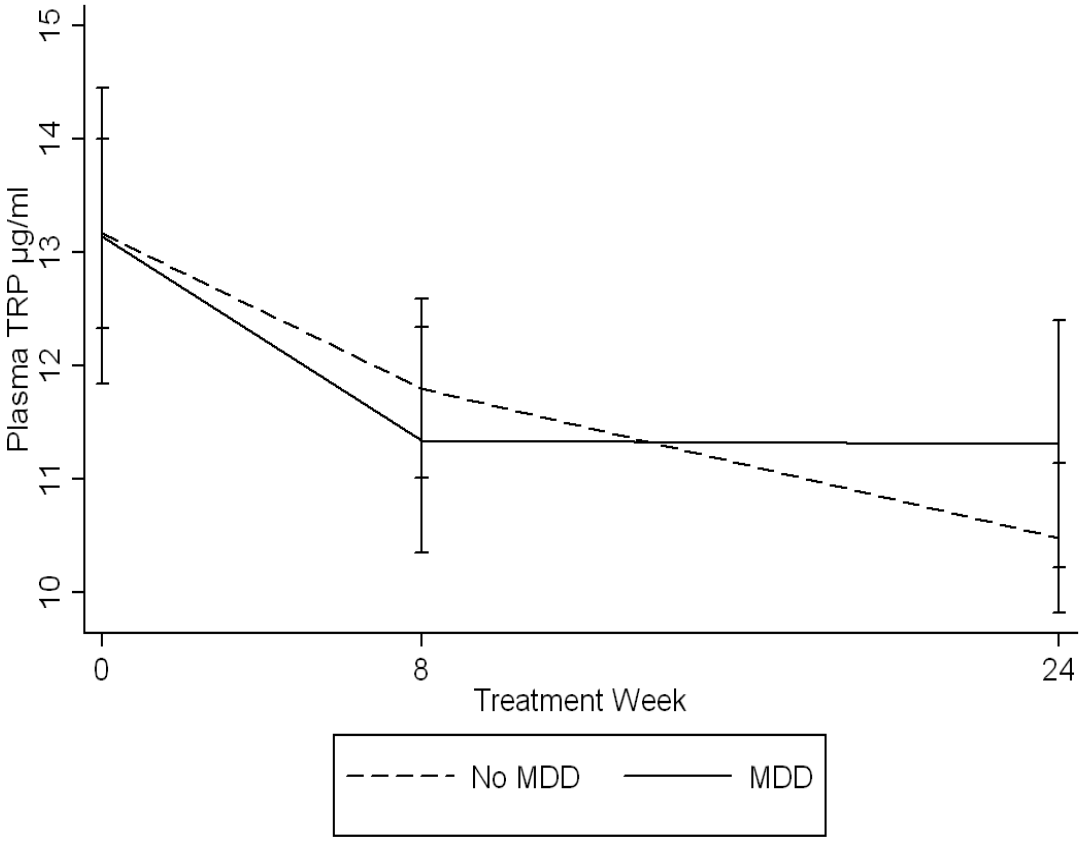


Figure 3.52 Changes in tryptophan levels in patients with and without IFN-α-induced depression

Changes in plasma levels of tryptophan (TRP) in patients with (*n* ranging from 16-21) and without (*n* ranging from 12-17) IFN-α-induced depression, across the 24 weeks of treatment.

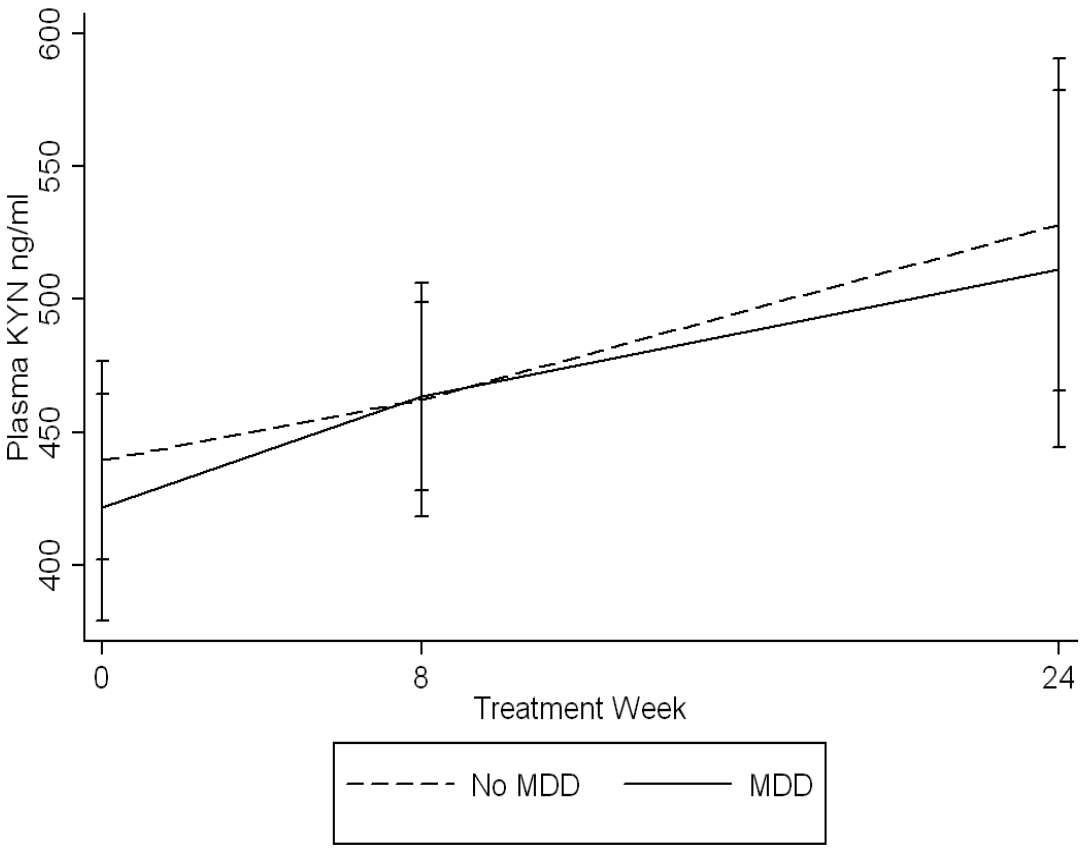


Figure 3.53 Changes in kynurenine levels in patients with and without IFN-α-induced depression

Changes in plasma levels of kynurenine (KYN) in patients with (*n* ranging from 16-21) and without (*n* ranging from 12-17) IFN-α-induced depression, across the 24 weeks of treatment.

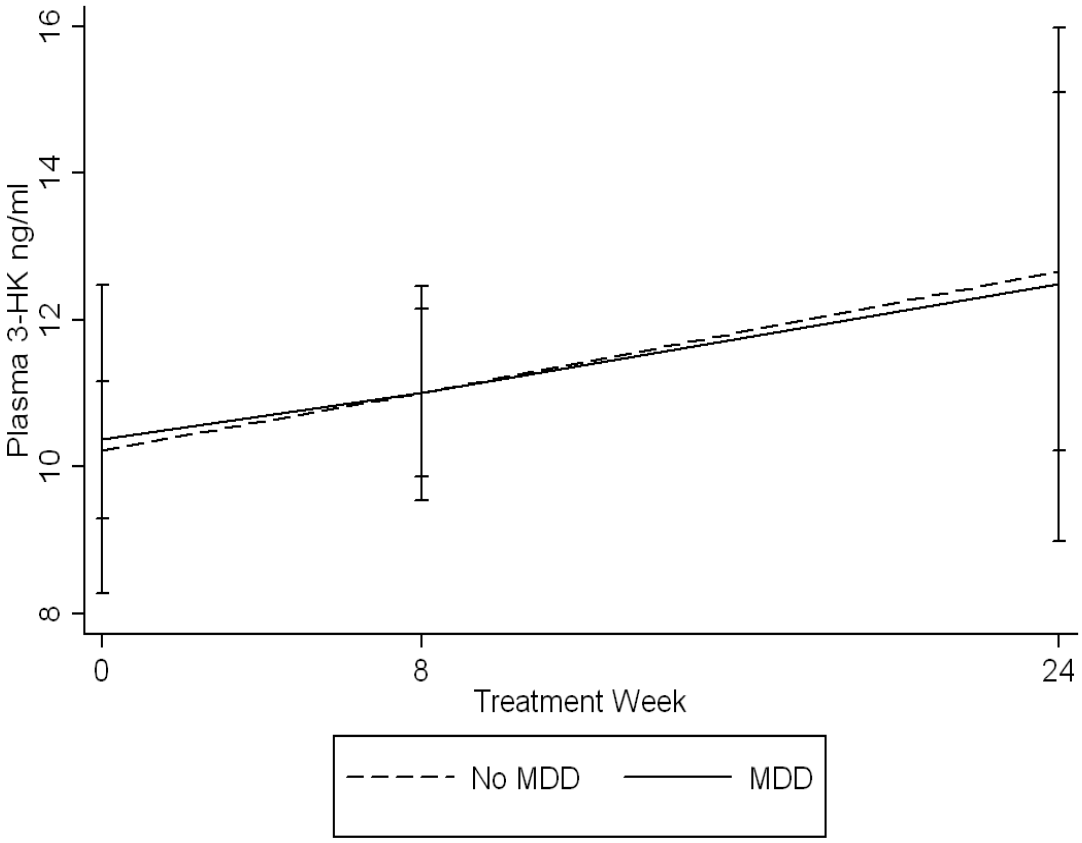


Figure 3.54 Changes in 3-hydroxykynurenine levels in patients with and without IFN-α-induced depression

Changes in plasma levels of 3-hydroxykynurenine (3-HK) in patients with (*n* ranging from 16-21) and without (*n* ranging from 12-17) IFN-α-induced depression, across the 24 weeks of treatment.

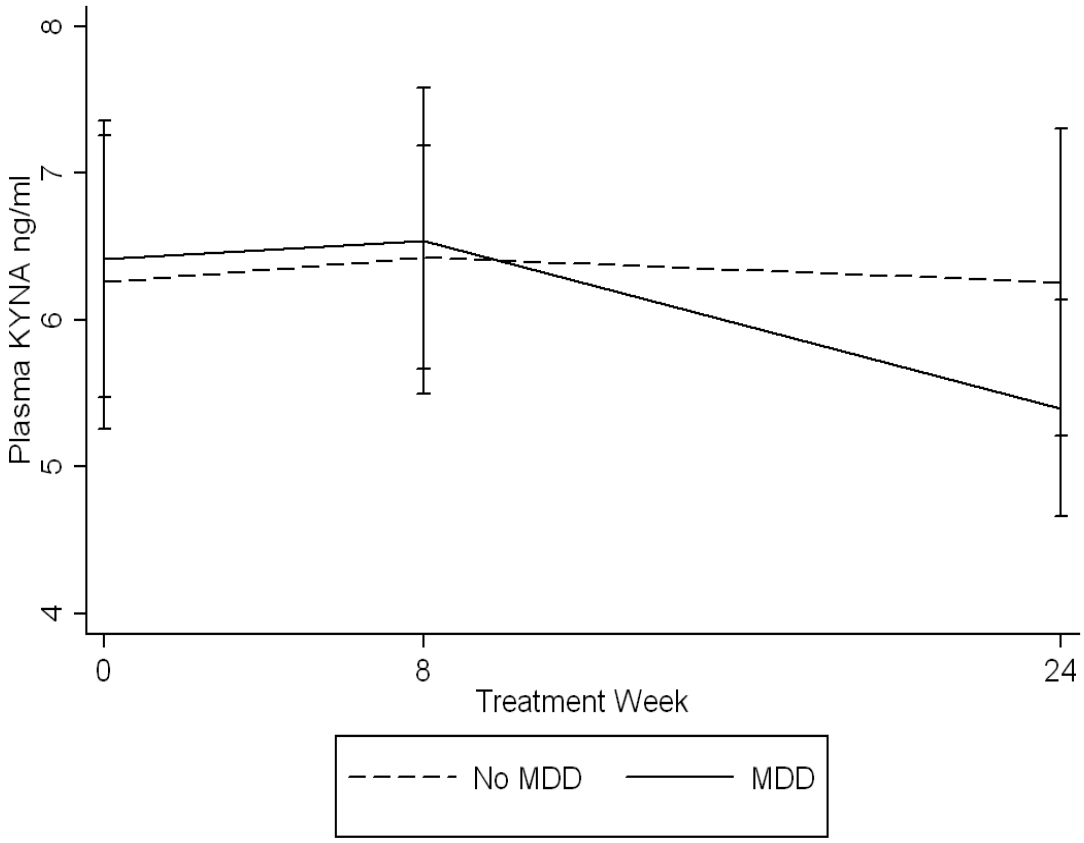


Figure 3.55 Changes in kynurenic acid levels in patients with and without IFN-α-induced depression

Changes in plasma levels of kynurenic acid (KYNA) in patients with (*n* ranging from 16-21) and without (*n* ranging from 12-17) IFN-α-induced depression, across the 24 weeks of treatment.

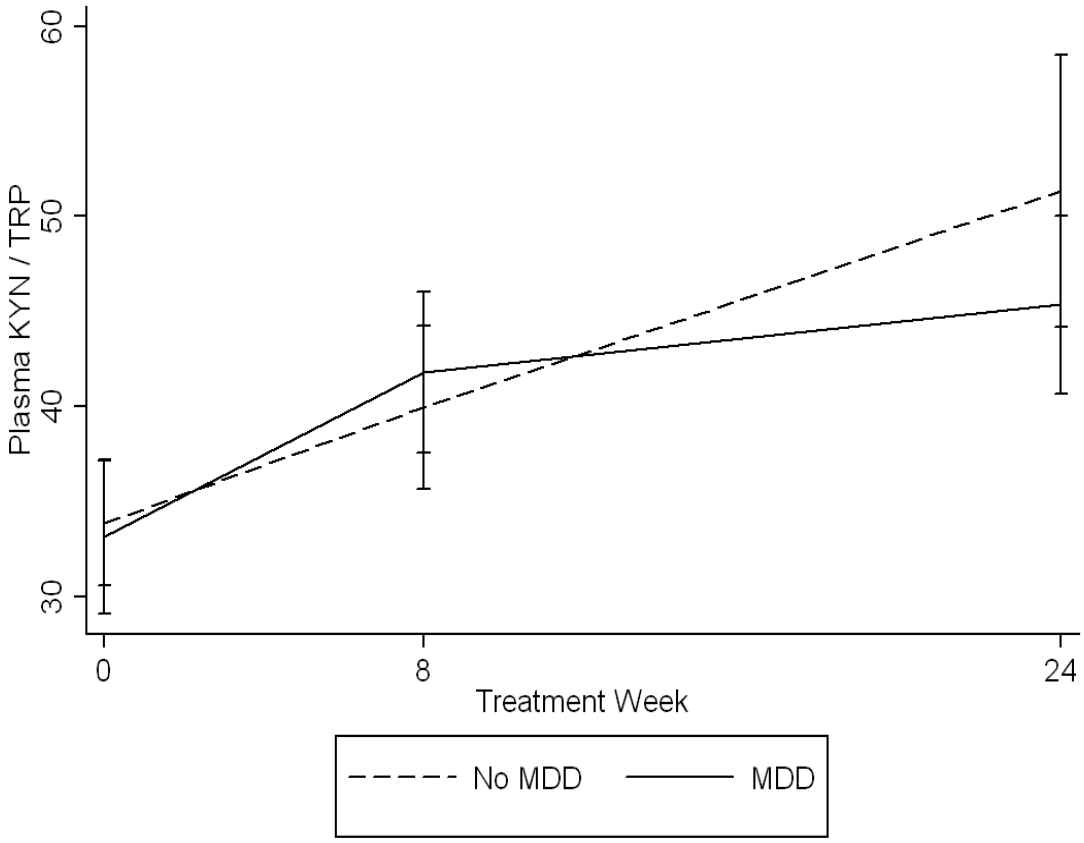


Figure 3.56 Changes in the kynurenine/tryptophan ratio in patients with and without IFN-α-induced depression

Changes in the kynurenine/tryptophan ratio (KYN/TRP) in patients with (*n* ranging from 16-21) and without (*n* ranging from 12-17) IFN-α-induced depression, across the 24 weeks of treatment.

3.1.7.3 Polyunsaturated fatty acids (PUFAs)

The differences in baseline PUFA levels in patients with and without IFN-α-induced depression are shown in Table 3.18. There were no significant differences between the two groups for any of the PUFAs. Changes in PUFA levels in patients with and without IFN-α-induced depression during the treatment period are shown in Figure 3.57-Figure 3.62. There was no significant effect of depression status on EPA, DHA or ALA levels ($p=0.8$, $p=0.9$ and $p=0.2$ respectively). As mentioned earlier, the two omega-6 PUFAs that were measured – AA and LA, both appeared to decrease during IFN-α treatment. However, there was no significant effect of depression status on AA or LA levels ($p=0.3$ and $p=0.1$, respectively). Finally, there was no significant effect of depression status on the ratio of omega-6 to omega-3 PUFAs ($p=0.4$). Independent samples t-tests also confirmed that there were no significant differences between patients with and without IFN-α-induced depression for any of the PUFAs measured, at any time point during IFN-α treatment.

Table 3.18 Baseline PUFA levels of patients with and without IFN-α-induced depression

	MDD ***n* = 19**	**No MDD** ***n* = 26**	
EPA	0.7±0.1	0.6±0.1	*t*=0.2, df=43, p=0.4
DHA	1.5±0.2	1.5±0.2	*t*=1.4, df=43, p=0.8
ALA	0.7±0.1	0.7±0.1	*t*=0.8, df=43, p=1.0
AA	5.6±0.3	6.1±0.3	*t*=-1.2, df=43, p=0.3
LA	26.1±0.9	27.6±0.9	*t*=0.5, df=43, p=0.3
AA/(EPA+DHA) ratio	3.2±0.4	3.8±0.4	*t*=1.0, df=43, p=0.3

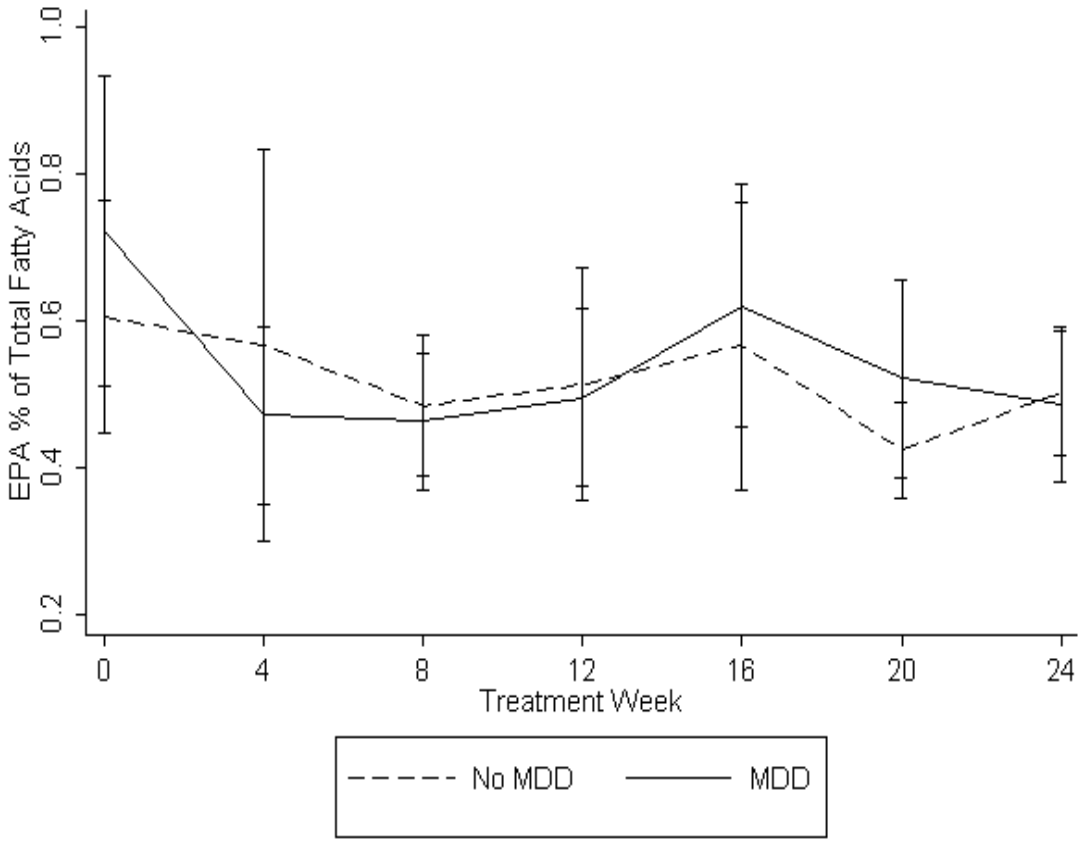


Figure 3.57 Changes in EPA levels in patients with and without IFN-α-induced depression

Changes in plasma levels of the of the omega-3 PUFA, eicosapentaenoic acid (EPA) in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

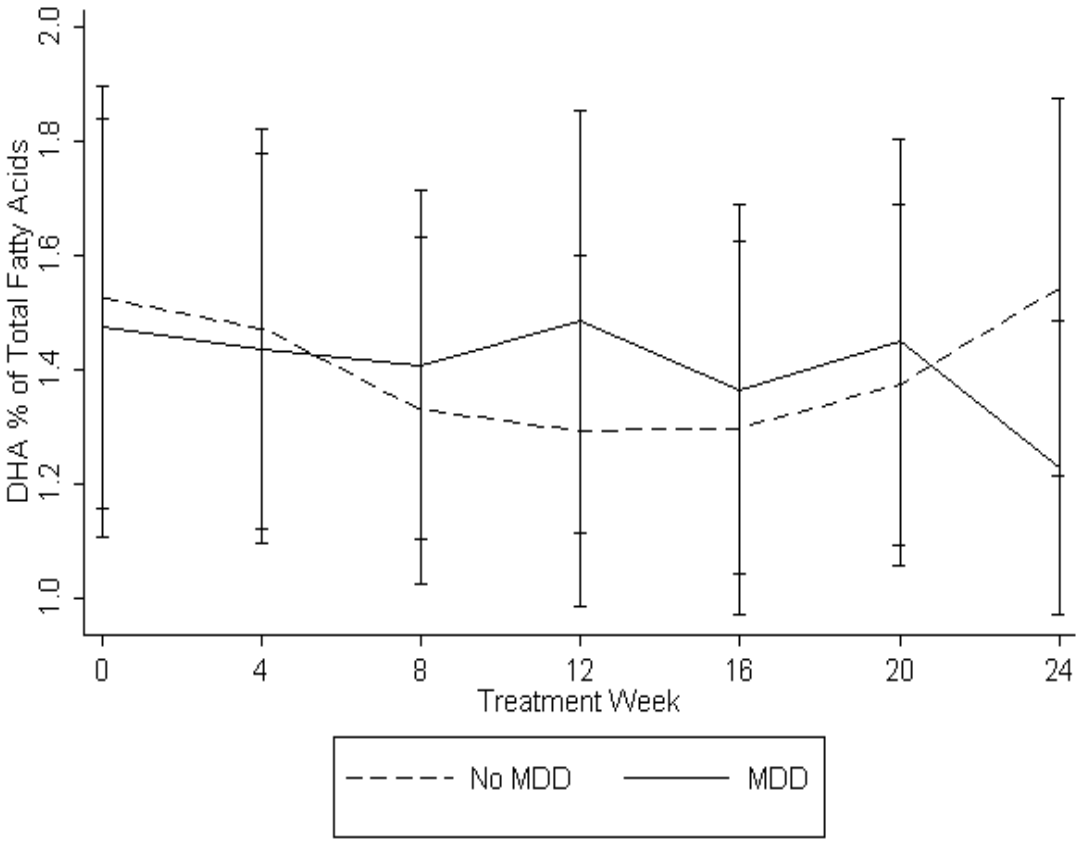


Figure 3.58 Changes in DHA levels in patients with and without IFN-α-induced depression

Changes in plasma levels of the omega-3 PUFA, docosahexaenoic acid (EPA) in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

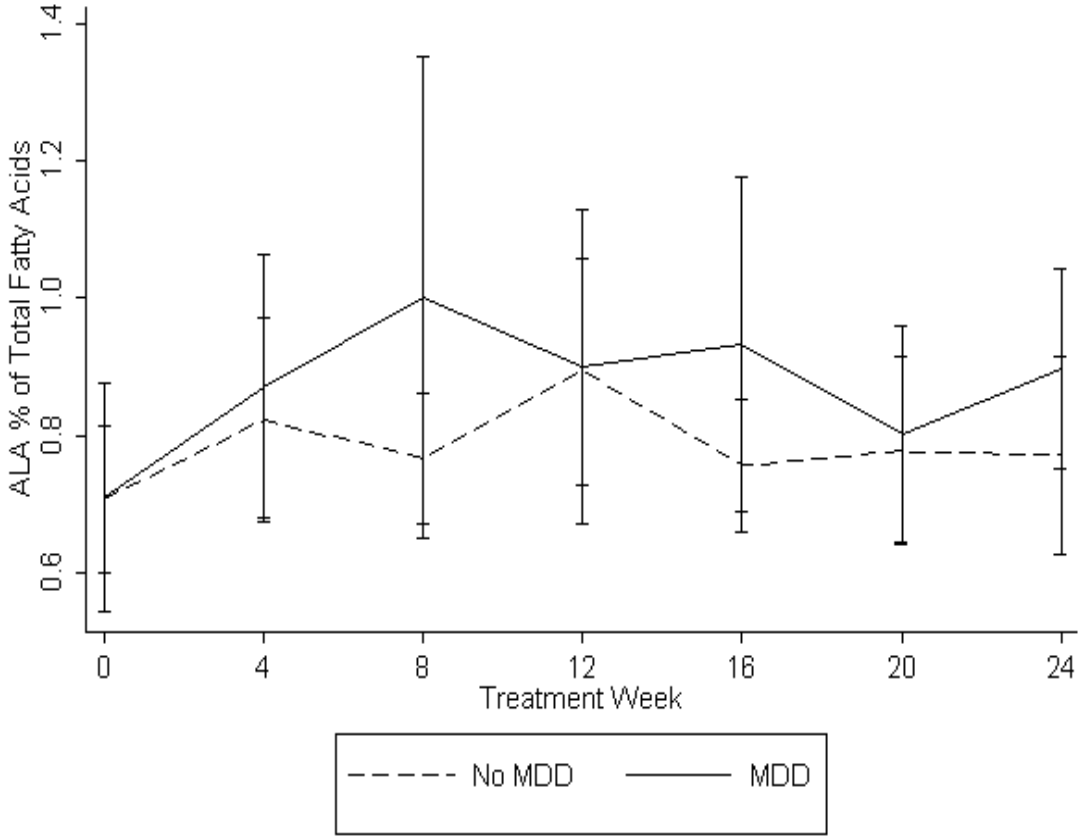


Figure 3.59 Changes in ALA levels in patients with and without IFN-α-induced depression

Changes in plasma levels of the omega-3 PUFA, alpha-linolenic acid (ALA) in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

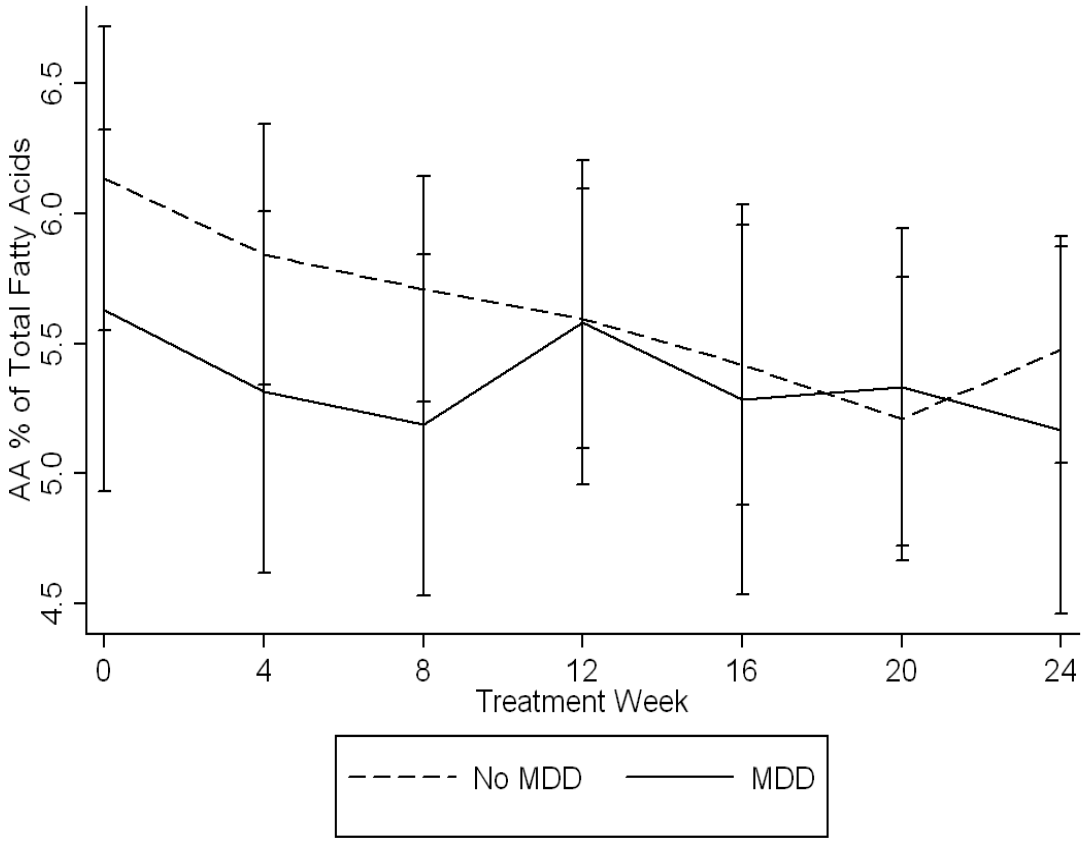


Figure 3.60 Changes in AA levels in patients with and without IFN-α-induced depression

Changes in plasma levels of the omega-6 PUFA, arachidonic acid (AA) in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

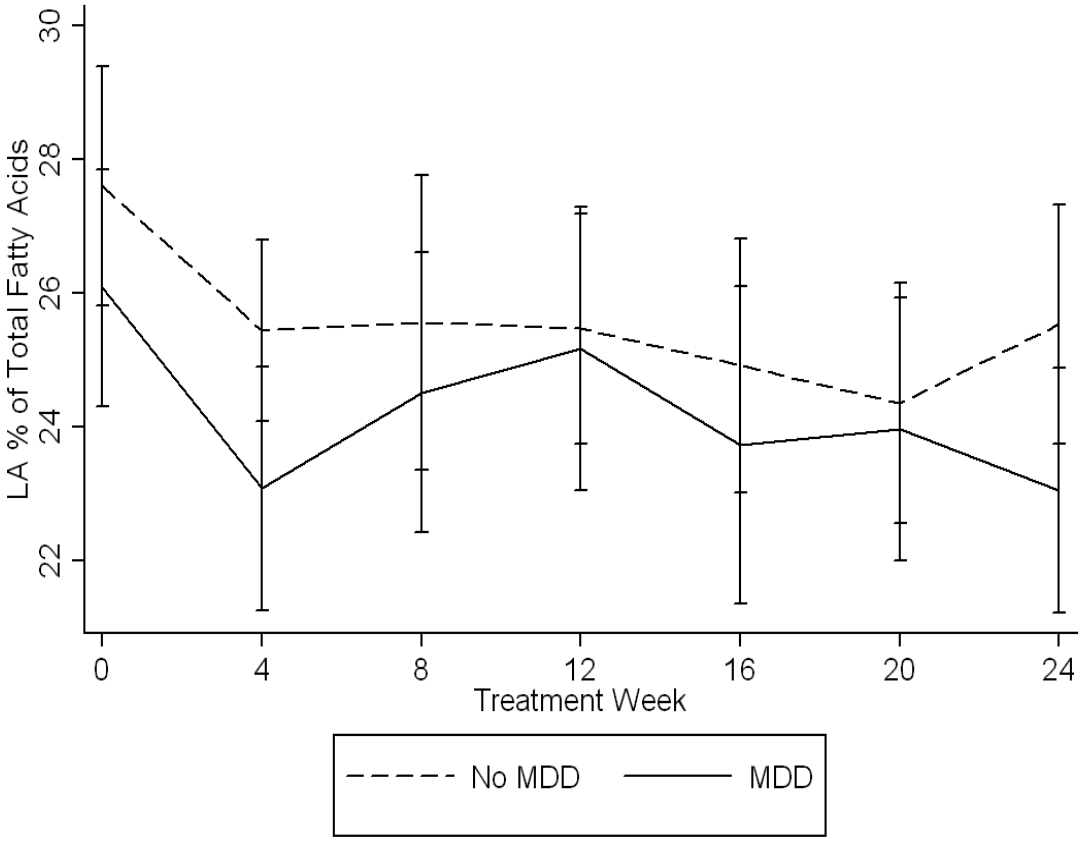


Figure 3.61 Changes in LA levels in patients with and without IFN-α-induced depression

Changes in plasma levels of the omega-6 PUFA, linoleic acid (LA) in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

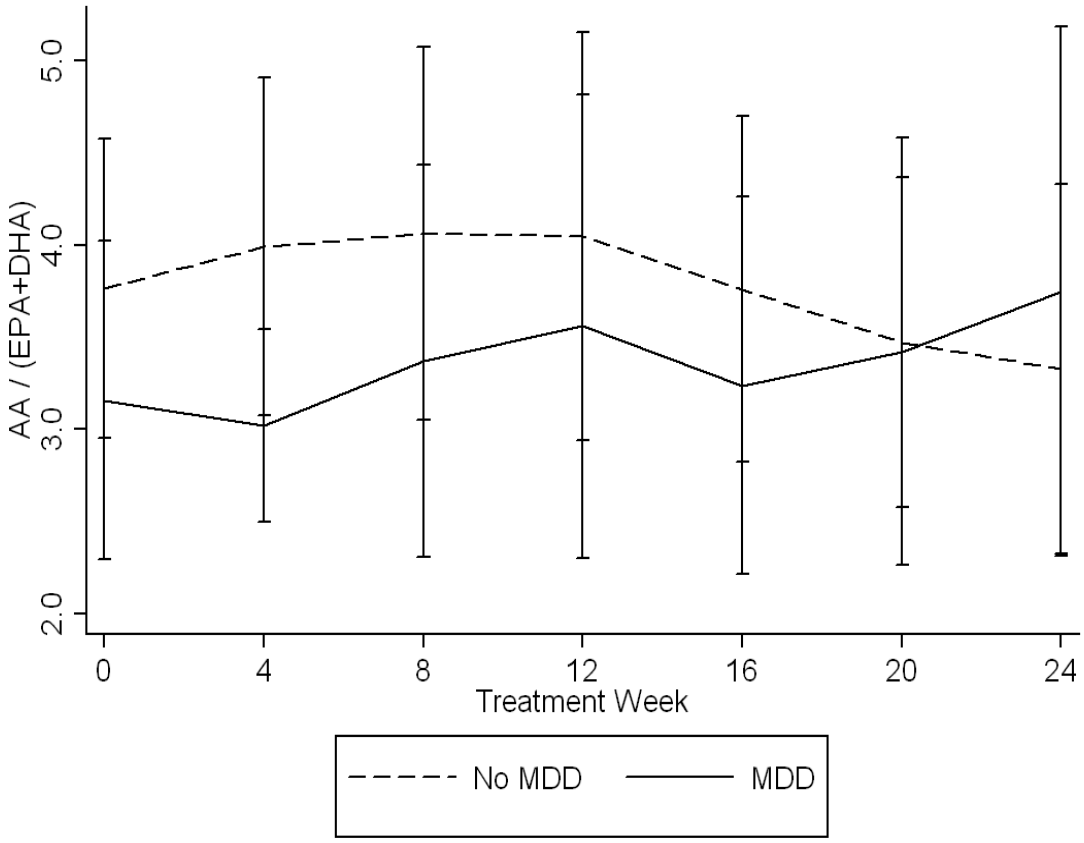


Figure 3.62 Changes in the AA / (EPA+DHA) ratio in patients with and without IFN-α-induced depression

Changes in ratio of the omega-6 PUFA, arachidonic acid (AA), to the omega-3 PUFAs; eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in patients with (*n* ranging from 19-29) and without (*n* ranging from 15-19) IFN-α-induced depression, across the 24 weeks of treatment.

3.1.7.4 Gene expression

To examine differences in baseline gene expression in patients with and without IFN-α-induced depression, I firstly used a hypothesis-free approach, to identify genes with an absolute fold change of 1.4 and a p-value cut-off of $p<0.005$. Eight differentially expressed genes were identified and are presented in Table 3.19. Among these most significant differentially expressed genes, of interest there was glutathione S-transferase mu 4 (GSTM4), which plays an important role in detoxifying various toxicants such as reactive oxygen species, which are known to induce oxidative stress. At baseline there was a lower expression of GSTM4 in patients who later developed IFN-α-induced depression.

Table 3.19 Differentially expressed genes at baseline in patients who develop IFN-α-induced depression (*n*=19) compared to those who do not (*n*=27)

Gene Symbol	Gene Title	Fold Change	P Value
AIM2	Absent in melanoma 2	-1.6	0.003
CAPSL	Calcyphosine-like	1.7	0.003
DUX4L7	Double homeobox 4 like 7	1.8	0.004
ERICH1	Glutamate-rich 1	-1.4	0.004
GSTM4	Glutathione S-transferase mu 4	-1.6	0.001
NBEAL1	Neurobeachin-like 1	-1.4	<0.001
PNPT1	Polyribonucleotide nucleotidyltransferase 1	-1.4	0.005
RNF144B	Ring finger protein 144B	1.4	0.005

Secondly, using a hypothesis-driven approach, I explored differences in the baseline expression of candidate genes in patients with and without IFN-α-induced depression. These data are presented in Table 3.20 and Table 3.21. This included the same genes belonging to 4 interlinked domains relevant for the development of depression, as in the previous tables. Patients who developed IFN-α-induced depression had significantly lower expression of IDO1 and KYNU (p=0.021 and p=0.037, respectively). There were no significant differences between the two groups in their expression of genes related to PUFA metabolism. Patients who developed IFN-α-induced depression had significantly higher expression of interleukin-1 alpha (IL-1A), IL-4 and IL-28B at baseline when compared to patients without IFN-α-induced depression (p=0.022, p=0.018 and p=0.032, respectively). They also had significantly lower expression of TNF receptor associated factor 6 (TRAF6) at baseline when compared to patients who did not develop IFN-α-induced depression (p=0.020). Finally, patients who developed IFN-α-induced depression had significantly lower expression of NR3C1 (GR) (p=0.011).

Table 3.20 Differentially expressed candidate genes at baseline in patients who develop IFN-α-induced depression (*n*=19) compared to those who do not (*n*=27)

Gene Symbol	Gene Title	Fold Change	P Value
Tryptophan metabolism			
IDO1	Indoleamine 2,3-dioxygenase 1	-1.36	**0.021**
IDO2	Indoleamine 2,3-dioxygenase 2	1.02	0.5
KMO	Kynurenine 3-monooxygenase (kynurenine 3-hydroxylase)	-1.23	0.1
KYNU	Kynureninase	-1.21	**0.037**
HAAO	3-hydroxyanthranilate 3,4-dioxygenase	1.05	0.1
TDO2	Tryptophan 2,3-dioxygenase	1.06	0.1
TPH1	Tryptophan hydroxylase 1	1.01	0.8
PUFA metabolism			
COX1	Cyclooxygenase 1	1.01	0.6
COX2	Cyclooxygenase 2	-1.02	0.7
COX3	Cyclooxygenase 3	1.04	0.5
FADS1	Fatty acid desaturase 1	-1.01	0.9
FADS2	Fatty acid desaturase 2	1.08	0.7
PLA2G2A	Phospholipase A2, group II	1.03	0.4

Table 3.21 Differentially expressed candidate genes at baseline in patients who develop IFN-α-induced depression (*n*=19) compared to those who do not (*n*=27)

Gene Symbol	Gene Title	Fold Change	P Value
Inflammation			
IL-1A	Interleukin 1, alpha	1.06	**0.022**
IL-1B	Interleukin 1, beta	-1.04	0.7
IL-1R1	Interleukin 1 receptor, type 1	-1.24	0.1
IL-2	Interleukin 2	1.02	0.4
sIL-2R	Soluble interleukin 2 receptor	-1.07	1.0
IL-4	Interleukin 4	1.05	**0.018**
IL-6	Interleukin 6	1.02	0.6
IL-6R	Interleukin 6 receptor	1.01	0.9
IL-8	Interleukin 8	-1.15	0.3
IL-10	Interleukin 10	1.07	0.1
IL-18	Interleukin 18	-1.12	0.3
IL-28B	Interleukin 28, beta	1.15	**0.038**
IFNG	Interferon, gamma	1.02	0.7
TGFB1	Transforming growth factor, beta 1	1.00	0.9
TNFA	Tumor necrosis factor, alpha	-1.13	0.1
TRAF6	TNF receptor associated factor 6	-1.12	**0.020**
Neuroplasticity			
BDNF	Brain-derived neurotrophic factor	1.01	0.8
FKBP4	FK506 binding protein 4	-1.07	0.3
FKBP5	FK506 binding protein 5	1.02	0.8
NR3C1	Nuclear receptor subfamily 3, group C, member 1	-1.10	**0.011**
VEGFA	Vascular endothelial growth factor A	-1.01	0.8
VGF	VGF nerve growth factor inducible	1.09	0.1

Changes in gene expression in patients with and without IFN-α-induced depression from baseline to treatment week 4 of IFN-α treatment were investigated using an absolute fold change of 1.4 and a p-value cut-off of $p<0.05$. Firstly, when comparing the gene expression profile of patients with IFN-α-induced depression at treatment week 4 versus baseline, approximately 750 differentially expressed genes were obtained. This analysis was repeated in patients without IFN-α-induced depression only obtaining in this case approximately 400 differentially expressed genes at treatment week 4 when compared to baseline. Further investigation revealed 322 genes to be in common between the two groups of patients, whereas 442 genes were modulated by IFN-α only in patients with IFN-α-induced depression, and 46 genes were modulated by IFN-α only in patients without IFN-α-induced depression. These data are presented in Figure 3.63.

As the focus of this thesis was the development of depression, pathway analysis of the 442 genes modulated by IFN-α specifically in patients with IFN-α-induced depression was conducted. Eight pathways were found to be regulated: Phosphatidylinositol (PIP) signalling system, long term potentiation (LTP) signalling, gap junction and notch signalling pathways were all down-regulated. The Janus kinase-signal transducers and activators of transcription (JAK-STAT) signalling, natural killer cells mediated cell cytotoxicity, DNA replication and toll like receptor signalling pathways were all up-regulated.

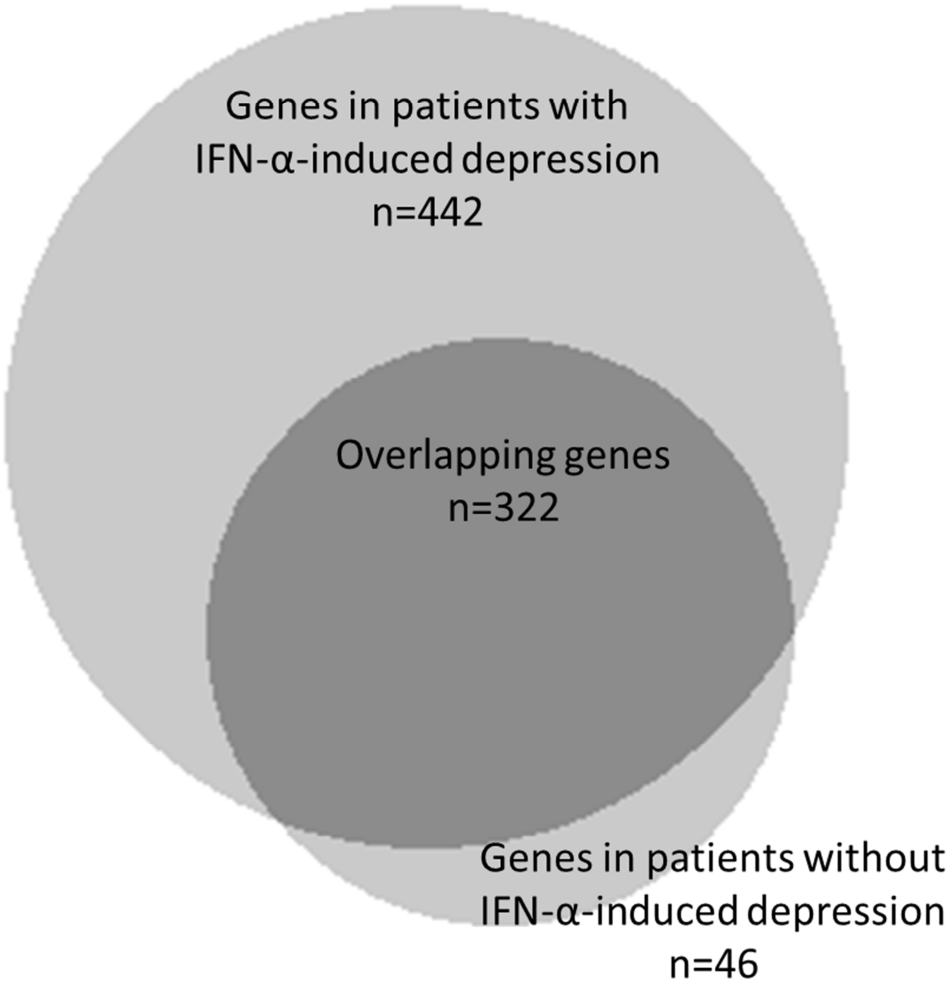


Figure 3.63 Venn diagram of genes modulated by IFN-α in patients with and without IFN-α-induced depression

Changes in the expression of candidate genes from baseline to treatment week 4, in patents with and without IFN-α-induced depression are presented in Table 3.22 and Table 3.23. Patients with IFN-α-induced depression had significantly lower expression of TDO2 at treatment week 4 when compared to baseline (p=0.002), whereas patients without IFN-α-induced depression had no change in TDO2 expression (p=0.6). Patients without IFN-α-induced depression also had a lower expression of IDO2 at treatment week 4 when compared to baseline (p=0.044) whereas this was unchanged in patients with IFN-α-induced depression (p=0.1).

Regarding genes related to PUFA metabolism, both patients with and without IFN-α-induced depression had significantly higher expression of FADS2 at treatment week 4 when compared to baseline (p=0.007 and p=0.029, respectively). Both patients with and without IFN-α-induced depression had significantly lower expression of COX1 at treatment week 4 when compared to baseline (p=0.001 and p=0.022, respectively). Finally, patients with IFN-α-induced depression had significantly lower expression of COX3 (p=0.019).

Table 3.22 Differentially expressed candidate genes at treatment week 4 compared to baseline in patients with and without IFN-α-induced depression

		MDD *n* = 19		No MDD *n* = 26	
Gene Symbol	**Gene Title**	**Fold Change**	**P Value**	**Fold Change**	**P Value**
Tryptophan metabolism					
IDO1	Indoleamine 2,3 dioxygenase 1	-1.15	0.4	-1.07	0.7
IDO2	Indoleamine 2,3 dioxygenase 2	-1.07	0.1	-1.08	**0.044**
KMO	Kynurenine 3 monooxygenase (kynurenine 3-hydroxylase)	1.17	0.2	1.01	0.9
KYNU	Kynureninase	1.11	0.4	-1.01	0.9
HAAO	3-hydroxyanthranilate 3,4-dioxygenase	-1.00	0.9	-1.07	0.1
TDO2	Tryptophan 2,3-dioxygenase	-1.08	**0.002**	1.01	0.6
TPH1	Tryptophan hydroxylase 1	1.01	0.9	1.03	0.6
PUFA metabolism					
COX1	Cyclooxygenase 1	-1.11	**0.001**	-1.07	**0.022**
COX2	Cyclooxygenase 2	-1.04	0.4	-1.06	0.2
COX3	Cyclooxygenase 3	-1.23	**0.019**	-1.17	0.1
FADS1	Fatty acid desaturase 1	1.11	0.2	1.14	0.1
FADS2	Fatty acid desaturase 2	1.61	**0.007**	1.46	**0.029**
PLA2G2A	Phospholipase A2, group II	1.06	0.1	1.01	0.7

Both patients with and without IFN-α-induced depression had significantly lower expression of IL-1B, IL-1R1 and IL-6R at treatment week 4 when compared to baseline ($p=0.001$; $p<0.001$; $p<0.001$ and $p<0.001$; $p<0.001$; $p<0.001$, respectively). Patients with IFN-α-induced depression also had significantly lower expression of IL-2 and IL-4 ($p=0.039$ and $p=0.001$, respectively). There was no change in the expression of these genes in patients without IFN-α-induced depression ($p=0.5$ and $p=0.9$ respectively). Patients with IFN-α-induced depression also had higher expression of interleukin-2 receptor gamma (IL-2RG), interleukin-18 (IL-18) and transforming growth factor beta-1 (TGFB1) ($p=0.021$, $p=0.006$ and $p=0.040$, respectively) at treatment week 4 when compared to baseline. There were no changes in the expression of these genes in patients without IFN-α-induced depression ($p=0.4$, $p=0.4$ and $p=0.1$, respectively). Furthermore, patients without IFN-α-induced depression had a higher expression of IL-10 at treatment week 4 when compared to baseline ($p=0.018$) whereas there was no significant change in IL-10 expression in patients with IFN-α-induced depression ($p=0.1$).

Finally, only patients without IFN-α-induced depression had lower expression of NR3C1 (GR) at treatment week 4 when compared to baseline ($p=0.002$). Neither patient group had any other significant changes in their expression of genes related to neuroplasticity.

Table 3.23 Differentially expressed candidate genes at treatment week 4 compared to baseline in patients with and without IFN-α-induced depression

		MDD ***n* = 19**		**No MDD** ***n* = 26**	
Gene Symbol	**Gene Title**	**Fold Change**	**P Value**	**Fold Change**	**P Value**
Inflammation					
IL-1A	Interleukin 1, alpha	-1.05	0.1	1.02	0.4
IL-1B	Interleukin 1, beta	-1.38	**0.001**	-1.44	**<0.001**
IL-1R1	Interleukin 1 receptor, type 1	-1.59	**<0.001**	-1.63	**<0.001**
IL-2	Interleukin 2	-1.05	**0.039**	1.01	0.5
sIL-2R	Soluble interleukin 2 receptor	1.08	0.5	1.05	0.6
IL-4	Interleukin 4	-1.08	**0.001**	-1.00	0.9
IL-6	Interleukin 6	-1.01	0.8	-1.02	0.5
IL-6R	Interleukin 6 receptor	-1.48	**<0.001**	-1.32	**<0.001**
IL-8	Interleukin 8	1.08	0.4	1.00	1.0
IL-10	Interleukin 10	1.10	0.1	1.13	**0.018**
IL-18	Interleukin 18	1.31	**0.006**	1.09	0.4
IL-28B	Interleukin 28, beta	-1.09	0.1	1.08	0.2
IFNG	Interferon, gamma	1.08	0.2	-1.02	0.8
TGFB1	Transforming growth factor, beta 1	1.12	**0.040**	-1.10	0.1
TNFA	Tumor necrosis factor, alpha	1.06	0.4	-1.01	0.9
TRAF6	TNF receptor associated factor, 6	1.06	0.3	-1.04	0.7
Neuroplasticity					
BDNF	Brain-derived neurotrophic factor	-1.03	0.3	-1.01	0.8
FKBP4	FK506 binding protein 4	1.03	0.5	-1.00	0.9
FKBP5	FK506 binding protein 5	1.05	0.6	1.09	0.3
NR3C1	Nuclear receptor subfamily 3, group C, member 1	-1.07	0.1	-1.13	**0.002**
VEGFA	Vascular endothelial growth factor A	-1.04	0.4	-1.05	0.3
VGF	VGF nerve growth factor inducible	-1.02	0.7	1.01	0.8

3.1.8 Clinical predictors of depression scores

Given that there were increases in depression scores in the whole sample, even in patients who did not meet criteria for MDD diagnosis, I investigated predictors of depression scores during IFN-α treatment as a continuous variable. In order to test the second and third primary aims of this thesis, I investigated six groups of predictors: socio-demographics, psychosocial stressors, cognitive factors, baseline psychopathology, baseline health status and biological factors.

The socio-demographic predictors are presented in Table 3.24. There were no socio-demographic factors that significantly predicted subsequent depression scores during IFN-α treatment. Furthermore, there were also no significant predictive effects of the liver disease parameters that were investigated.

The predictive effects of psychosocial stressors are presented in Table 3.25. There were no significant predictive effects of any of the psychosocial stressors on depression scores during IFN-α treatment.

Table 3.24 Socio-demographic predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Age	-0.1	0.2	0.7	-0.1	0.1	0.7
Gender	-4.9	4.4	0.3	-2.5	3.5	0.5
Ethnicity	-0.03	3.9	1.0	2.6	3.1	0.4
Education Level	3.1	5.8	0.6	6.2	4.5	0.2
Employment	-5.1	3.9	0.2	-5.8	3.1	0.1
Relationship Status	-0.3	3.8	0.9	0.03	3.1	1.0
History of MDD	6.8	3.9	0.1	1.5	3.3	0.6
Family History	1.7	4.4	0.7	-3.9	3.9	0.3
Substance Use	0.4	3.9	0.9	-0.04	3.3	1.0
Genotype	1.5	2.2	0.5	-0.2	1.8	0.9
Viral Load	-1.0	0.7	0.2	-0.5	0.5	0.3
Fibroscan	-0.1	0.3	0.9	0.04	0.2	0.8

Table 3.25 Psychosocial stress predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Any BLE	6.0	3.8	0.1	0.7	3.2	0.8
Parental Separation	-6.0	4.4	0.2	-4.4	3.5	0.2
Parental Loss	-2.1	6.3	0.7	2.3	5.1	0.6
Childhood Physical Abuse	-1.9	5.4	0.7	-3.5	4.3	0.4
Childhood Sexual Abuse	1.5	5.2	0.8	2.0	4.1	0.6
Any Childhood Trauma	-2.9	4.0	0.5	-0.8	3.3	0.8

The cognitive predictors (illness perceptions) are presented in Table 3.26. There was a significant effect of perceptions about timeline in predicting depression scores (Coefficient=1.0, p<0.001) and this remained significant after adjusting for baseline depression scores (p=0.032) and the overall model accounted for 31% of the variance (R^2=0.31). There were also significant effects of the consequences, timeline cyclical, personal control and emotional representations dimensions (Coefficient=0.9, p=0.003; Coefficient=1.5, p=0.002; Coefficient=-1.3, p=0.009 and Coefficient=0.9, p=0.001, respectively). However, all of these effects were no longer significant after adjusting for baseline depression scores (p=0.1, p=0.1, 0.2 and p=0.1, respectively) indicating these effects are in driven by baseline depression status.

Table 3.26 Cognitive predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Timeline	1.0	0.3	**<0.001**	0.6	0.3	**0.032**
Consequences	0.9	0.3	**0.003**	0.5	0.3	0. 1
Timeline Cyclical	1.5	0.5	**0.002**	0.8	0.5	0.1
Personal Control	-1.3	0.5	**0.009**	-0.6	0.5	0.2
Treatment Control	-0.5	0.4	0.2	-0.2	0.3	0.5
Illness Coherence	-0.3	0.5	0.5	0.2	0.4	0.6
Emotional Representations	0.9	0.3	**0.001**	0.5	0.3	0.1

The predictive effects of baseline psychopathology are presented in Table 3.27. There were significant effects of baseline scores of depression, fatigue, stress and anxiety on subsequent depression scores during IFN-α treatment (Coefficient=0.8, p<0.001; Coefficient=1.3, p<0.001; Coefficient=1.0, p<0.001 Coefficient=0.2 and p<0.001, respectively). However, after adjusting for baseline depression scores, none of these effects remained significant (p=0.2, p=0.9 and p=0.1, respectively) indicating that baseline depression scores drive the association between baseline fatigue, stress and anxiety scores, and subsequent depression scores.

All 8 health status dimensions of the SF-36 significantly predicted subsequent depression scores as presented in Table 3.28. After adjusting for baseline depression scores, significant negative effects of baseline scores remained for the emotional role limitation, social functioning and bodily pain dimensions (Coefficient=-0.2, R^2=0.36, p=0.046; Coefficient=-0.2, R^2=0.37, p=0.019 and Coefficient=-0.2, R^2=0.37, p=0.001, respectively). There were no longer any significant effects of baseline scores on the physical functioning, physical role limitation, vitality, mental health and general health dimensions (p=1.0, p=0.9, p=0.6, p=0.1 and p=0.1 respectively) indicating that these effects were driven by baseline depression status.

Table 3.27 Baseline psychopathology predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
IDS	0.8	0.1	**<0.001**	-	-	-
CFQ	1.3	0.4	**<0.001**	0.3	0.2	0.2
PSS	1.0	0.2	**<0.001**	-0.1	0.6	0.9
HADS-A	0.2	0.4	**<0.001**	-0.2	0.1	0.1

Table 3.28 Baseline health status predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Physical functioning	-0.4	0.1	**0.001**	<-0.01	0.05	1.0
Physical role limitation	-0.1	0.1	**0.024**	0.01	0.05	0.9
Emotional role limitation	-0.1	0.1	**0.032**	-0.2	0.1	**0.046**
Vitality	-0.3	0.1	**<0.001**	-0.1	0.1	0.6
Mental health	-0.4	0.1	**<0.001**	-0.1	0.1	0.1
Social functioning	-0.2	0.1	**<0.001**	-0.2	0.1	**0.019**
Bodily pain	-0.3	0.1	**<0.001**	-0.2	0.1	**0.001**
General health	-0.3	0.1	**<0.001**	-0.2	0.1	0.1

3.1.9 Biological predictors of depression scores

The biological predictors of depression scores during IFN-α treatment are presented in Table 3.29. After adjusting for baseline depression scores, there was a significant negative effect of the baseline AUC of cortisol during the day as well as the noon cortisol values in predicting depression scores (Coefficient=-<0.01, R^2=0.57, p=0.006 and Coefficient=-2.2, R^2=0.58, p=0.006, respectively). These data suggest the association between baseline cortisol activity and subsequent depression scores are mediated by baseline depression scores.

There was only a significant negative effect of kynurenic acid levels in predicting subsequent depression scores (Coefficient=-2.2, p=0.010). This remained significant after adjusting for baseline depression scores (p=0.035) and the overall model accounted for 44% of the variance (R^2=0.44).

There was no effect of baseline levels of any of the PUFAs measured. However, after adjusting for baseline depression scores, there was a significant negative effect of the omega-6 PUFA, arachidonic acid (AA) in predicting subsequent depression scores (Coefficient=-2.2, R^2=0.37, p=0.032).

Table 3.29 Biological predictors of depression scores during IFN-α treatment

	Post baseline IDS scores			Post baseline IDS scores adjusting for baseline IDS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Awakening AUCi	0.03	0.02	0.1	0.02	0.01	0.1
Delta 15 minutes	1.0	0.8	0.2	0.9	0.6	0.1
Delta 30 minutes	1.1	0.8	0.2	1.0	0.5	0.1
Delta 60 minutes	0.9	0.6	0.2	0.3	0.5	0.5
Day AUC	-<0.01	<0.01	0.3	-<0.01	<0.01	**0.006**
Noon	-0.9	1.3	0.5	-2.2	0.8	**0.006**
8PM	1.1	1.6	0.5	-0.9	1.2	0.4
Tryptophan	-0.1	0.8	1.0	1.2	0.6	0.1
Kynurenine	-0.02	0.02	0.3	-0.01	0.02	0.6
3-Hydroxykynurenine	0.2	0.6	0.8	0.2	0.4	0.6
Kynurenic acid	-2.2	0.9	**0.010**	-1.4	0.7	**0.035**
Kynurenine/Tryptophan	-0.2	0.2	0.4	-0.3	0.2	0.1
EPA	3.5	4.7	0.4	1.2	3.7	0.7
DHA	0.8	2.3	0.7	0.5	1.8	0.8
ALA	-2.2	6.5	0.7	1.8	5.3	0.7
AA	-1.1	1.3	0.4	-2.2	1.0	**0.032**
LA	-0.6	0.5	0.2	-0.5	0.4	0.2
AA/DHA + EPA	-0.6	1.0	0.6	-0.5	0.8	0.5

3.1.10 Clinical predictors of fatigue scores

The socio-demographic predictors of fatigue scores during IFN-α treatment are presented in Table 3.30. There was a significant negative effect of ethnicity, specifically being of a non-white British ethnicity (Coefficient=-3.0, p=0.038). This effect remained significant after adjusting for baseline fatigue scores (p=0.025) and the overall model accounted for 21% of the variance (R^2=0.21). There were no other socio-demographic factors that significantly predicted fatigue scores during treatment. Furthermore, there were also no significant predictive effects of the liver disease parameters that were investigated.

The predictive effects of psychosocial stressors are presented in Table 3.31. There was a significant predictive effect of having experienced a stressful life event (Coefficient=3.0, p=0.035). However, this was no longer significant after adjusting for baseline fatigue scores (p=0.4) indicating that this effect is driven by baseline fatigue scores.

Table 3.30 Socio-demographic predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Age	-0.04	0.1	0.6	-0.01	0.1	0.9
Gender	-1.6	1.7	0.4	-0.1	1.7	0.9
Ethnicity	-3.0	1.4	**0.038**	-2.6	1.3	**0.025**
Education Level	-0.6	2.3	0.8	0.3	2.0	0.9
Employment	-1.0	1.6	0.5	-1.2	1.4	0.4
Relationship Status	-0.4	1.5	0.8	-0.2	1.4	0.9
History of MDD	1.5	1.5	0.3	0.5	1.5	0.7
Family History	1.7	4.4	0.7	-3.9	3.9	0.3
Substance Use	0.5	1.6	0.8	1.0	1.4	0.5
Genotype	1.3	0.9	0.1	0.2	0.9	0.8
Viral Load	-0.1	0.3	0.7	0.04	0.2	0.9
Fibroscan	0.02	0.1	0.8	-0.01	0.1	0.9

Table 3.31 Psychosocial stress predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Any BLE	3.0	1.4	**0.035**	1.2	1.5	0.4
Parental Separation	0.9	1.6	0.6	2.5	1.6	0.1
Parental Loss	1.0	2.7	0.7	1.2	2.1	0.6
Childhood Physical Abuse	0.7	2.0	0.7	2.2	1.9	0.2
Childhood Sexual Abuse	3.0	1.8	0.1	3.1	1.8	0.1
Any Childhood Trauma	1.5	1.4	0.3	2.7	1.4	0.1

The cognitive predictors (illness perceptions) of fatigue scores are presented in Table 3.32. There was a significant effect of the consequences dimension (Coefficient=0.4, p=0.002). This effect remained significant after adjusting for baseline fatigue scores (p=0.031) and the overall model accounted for 11% of the variance (R^2=0.11). There was a significant effect of the emotional representations dimension (Coefficient=0.3, R^2=0.11, p=0.003). This effect also remained significant after adjusting for baseline fatigue scores (p=0.020) and the overall model accounted for 11% of the variance (R^2=0.11). There was also a significant effect of perceptions about timeline in predicting fatigue scores (Coefficient=0.3, p=0.005) however; this was no longer significant after adjusting for baseline fatigue scores (p=0.1) indicating that this effect was driven by baseline fatigue scores.

Table 3.32 Cognitive predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Timeline	0.3	0.1	**0.005**	0.2	0.1	0.1
Consequences	0.4	0.1	**0.002**	0.3	0.1	**0.031**
Timeline Cyclical	0.4	0.2	0.1	0.3	0.2	0.2
Personal Control	-0.1	0.2	0.7	0.1	0.2	0.8
Treatment Control	-0.2	0.2	0.2	-0.1	0.2	0.6
Illness Coherence	0.02	0.2	0.9	0.1	0.2	0.5
Emotional Representations	0.3	0.1	**0.003**	0.3	0.1	**0.020**

The predictive effects of baseline psychopathology are presented in Table 3.33. There were significant effects of baseline depression, fatigue, stress and anxiety scores on subsequent fatigue scores during IFN-α treatment (Coefficient=0.2, p=0.001; Coefficient=0.6, p=0.001; Coefficient=0.3, p=0.002 and Coefficient=0.6, p<0.001, respectively). However, after adjusting for baseline fatigue scores, none of these effects remained significant (p=0.9, p=0.4 and p=0.3, respectively) indicating that baseline fatigue scores were driving these associations.

There were significant effects for baseline scores on 6 out of the 8 health status dimensions including, physical functioning, physical role limitation, vitality, mental health, bodily pain and general health (Coefficient=-0.1, p=0.018; Coefficient=-0.04, p=0.026; Coefficient=-0.1, p<0.001; Coefficient=-0.1, p=0.004; Coefficient=-0.1, p=0.008 and Coefficient=-0.1, p=0.001, respectively). However, after adjusting for baseline fatigue scores, there was only a significant negative effect of baseline scores on the general health dimension (Coefficient=-0.1, R^2=0.21, p=0.044).

Table 3.33 Baseline psychopathology predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
IDS	0.2	0.1	**0.001**	-0.03	0.5	0.9
CFQ	0.6	0.2	**0.001**	-	-	-
PSS	0.3	0.1	**0.002**	0.1	0.1	0.4
HADS-A	0.6	0.2	**<0.001**	0.1	0.1	0.3

Table 3.34 Baseline health status predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Physical functioning	-0.1	0.1	**0.018**	-0.1	0.1	0.2
Physical role limitation	-0.04	0.02	**0.026**	-0.01	0.02	0.4
Emotional role limitation	-0.04	0.02	0.1	-0.01	0.02	0.7
Vitality	-0.1	0.03	**<0.001**	-0.1	0.04	0.1
Mental health	-0.1	0.04	**0.004**	-0.1	0.1	0.2
Social functioning	-0.1	0.03	0.1	-0.02	0.02	0.3
Bodily pain	-0.1	0.03	**0.008**	-0.04	0.03	0.3
General health	-0.1	0.03	**0.001**	-0.1	0.03	**0.044**

3.1.11 Biological predictors of fatigue scores

The biological predictors of fatigue scores during IFN-α treatment are presented in Table 3.35. There was no significant effect of cortisol activity on subsequent fatigue scores. However, after adjusting for baseline fatigue scores, there was a significant negative effect of the baseline AUC of cortisol during the day as well as noon cortisol values in predicting fatigue scores during treatment (Coefficient=-<0.01, R^2=0.32, p=0.001 and Coefficient=-1.2, R^2=0.24, p=0.014, respectively).

There was only a significant negative effect of kynurenic acid levels in predicting subsequent fatigue scores (Coefficient=-1.0, p=0.002). This effect remained significant after adjusting for baseline fatigue scores (p=0.019) and the overall model accounted for 24% of the variance (R^2=0.24).

There were no significant predictive effects for any of the five PUFAs that were examined.

Table 3.35 Biological predictors of fatigue scores during IFN-α treatment

	Post baseline CFQ scores			Post baseline CFQ scores adjusting for baseline CFQ scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Awakening AUCi	0.01	0.01	0.4	<0.01	0.01	0.5
Delta 15 minutes	0.1	0.3	0.7	0.1	0.3	0.7
Delta 30 minutes	0.4	0.3	0.2	0.3	0.3	0.3
Delta 60 minutes	0.1	0.3	0.8	-0.04	0.3	0.9
Day AUC	-<0.01	<0.01	0.1	-<0.01	<0.01	**0.001**
Noon	-0.6	0.5	0.2	-1.2	0.5	**0.014**
8PM	-0.3	0.6	0.6	-0.53	0.6	0.4
Tryptophan	0.2	0.3	0.5	0.1	0.3	0.7
Kynurenine	-0.01	0.01	0.2	-0.01	0.01	0.1
3-Hydroxykynurenine	-0.2	0.2	0.4	-0.2	0.2	0.3
Kynurenic acid	-1.0	0.3	**0.002**	-0.8	0.3	**0.019**
Kynurenine/Tryptophan	-0.2	0.1	0.1	-0.2	0.1	0.1
EPA	0.1	1.8	0.9	0.7	1.6	0.7
DHA	0.1	0.9	0.9	0.4	0.8	0.6
ALA	-0.9	2.5	0.7	-1.2	2.3	0.6
AA	0.04	0.5	0.9	0.1	0.5	0.9
LA	-0.3	0.2	0.1	-0.2	0.2	0.2
AA/DHA + EPA	-0.1	0.4	0.8	-0.1	0.3	0.8

3.1.12 Clinical predictors of stress scores

The socio-demographic predictors of stress scores during IFN-α treatment are presented in Table 3.36. There was a large significant effect of having a previous history of MDD (Coefficient=7.6, p<0.001). This effect remained significant after adjusting for baseline stress scores (p=0.006) and the overall model accounted for 43% of the variance (R^2=0.43). There were no other socio-demographic factors that significantly predicted fatigue scores during treatment. Furthermore, there were also no significant predictive effects of the liver disease parameters that were investigated.

The predictive effects of psychosocial stressors are presented in Table 3.37. There was a significant predictive effect of having experienced a stressful life event on stress scores during IFN-α treatment (Coefficient=4.4, p=0.048). However, this effect was no longer significant after adjusting for baseline stress scores (p=0.4) indicating that this association is driven by baseline stress scores. There were no other significant predictive effects of psychosocial stressors on subsequent stress scores.

Table 3.36 Socio-demographic predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Age	-0.1	0.1	0.1	-0.1	0.1	0.1
Gender	-2.5	2.6	0.4	0.3	2.0	0.9
Ethnicity	-1.5	2.3	0.5	0.2	1.7	0.9
Education Level	-0.1	3.5	1.0	2.8	2.5	0.3
Employment	-2.5	2.4	0.3	-0.4	1.8	0.8
Relationship Status	-0.2	2.3	0.9	0.03	1.7	1.0
History of MDD	7.6	2.2	**<0.001**	4.7	1.7	**0.006**
Family History	2.4	2.6	0.4	0.5	2.0	0.8
Substance Use	-0.02	2.4	1.0	0.5	1.7	0.8
Genotype	1.7	1.3	0.2	0.7	1.0	0.4
Viral Load	-0.5	0.4	0.2	-0.1	0.3	0.8
Fibroscan	-0.01	0.2	1.0	-0.1	0.1	0.5

Table 3.37 Psychosocial stress predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Any BLE	4.4	2.2	**0.048**	1.6	1.7	0.4
Parental Separation	-3.7	2.5	0.1	-2.7	1.9	0.1
Parental Loss	1.3	3.6	0.7	0.9	2.7	0.7
Childhood Physical Abuse	-1.0	3.2	0.8	-2.0	2.3	0.4
Childhood Sexual Abuse	0.3	3.0	0.9	-2.2	2.3	0.3
Any Childhood Trauma	-1.3	2.3	0.6	-1.9	1.7	0.3

The cognitive predictors (illness perceptions) are presented in Table 3.38. There was a significant effect of the emotional representations dimension (Coefficient=0.7, p<0.001). This effect remained significant after adjusting for baseline stress scores (p=0.043) and the overall model accounted for 36% of the variance (R^2=0.36). There was also significant effects of perceptions about timeline, consequences, timeline cyclical dimension, personal control and illness coherence in predicting stress scores (Coefficient=0.7, p<0.001; Coefficient=0.7, p<0.001; Coefficient=1.3, p<0.001; Coefficient=-1.1, p<0.001 and Coefficient=-0.6, p=0.046, respectively). However, all of these effects were no longer significant after adjusting for baseline stress scores (p=0.2; p=0.1; p=0.1, p=0.3 and p=0.6, respectively) indicating that these associations are driven by baseline stress scores.

Table 3.38 Cognitive predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Timeline	0.7	0.2	**<0.001**	0.2	0.2	0.2
Consequences	0.7	0.2	**<0.001**	0.3	0.2	0.1
Timeline Cyclical	1.3	0.3	**<0.001**	0.5	0.3	0.1
Personal Control	-1.1	0.3	**<0.001**	-0.3	0.3	0.3
Treatment Control	-0.3	0.2	0.1	0.2	0.2	0.4
Illness Coherence	-0.6	0.3	**0.046**	0.1	0.2	0.6
Emotional Representations	0.7	0.2	**<0.001**	0.3	0.2	**0.043**

The predictive effects of baseline psychopathology are presented in Table 3.39. There were significant effects of baseline depression, fatigue and anxiety scores on subsequent stress scores during IFN-α treatment (Coefficient=0.4, p<0.001; Coefficient=1.0, p<0.001; Coefficient=1.2 p<0.001, respectively). After adjusting for baseline stress scores, there was still a significant effect of baseline depression and anxiety scores (Coefficient=0.2, R^2=0.41, p=0.047 and Coefficient=0.6, R^2=0.41, p=0.041, respectively) indicating that these affects are largely independent of baseline stress scores.

There were significant negative effects of baseline scores on all 8 health status dimensions, on subsequent stress scores during IFN-α treatment. These data are presented in Table 3.40. After adjusting for baseline stress scores, significant effects remained for the vitality, mental health and general health dimensions (Coefficient=-0.1, R^2=0.41, p=0.041, Coefficient=-0.1, R^2=0.42, p=0.045 and Coefficient=-0.1, R^2=0.43, p=0.005, respectively). The effects for physical functioning, physical role limitation, emotional role limitation, social functioning and bodily pain were no longer significant (p=1.0, p=0.8, p=0.1, p=0.5 and p=0.3, respectively), indicating that these effects were driven by baseline stress scores.

Table 3.39 Baseline psychopathology predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
IDS	0.4	0.1	**<0.001**	0.2	0.1	**0.047**
CFQ	1.0	0.2	**<0.001**	0.5	0.2	0.1
PSS	0.8	0.1	0.1	-	-	-
HADS-A	1.2	0.3	**<0.001**	0.6	0.3	**0.041**

Table 3.40 Baseline health status predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Physical functioning	-0.2	0.1	**0.009**	<-0.01	0.1	1.0
Physical role limitation	-0.1	0.03	**0.007**	-0.01	0.02	0.8
Emotional role limitation	-0.1	0.03	**0.001**	-0.04	0.02	0.1
Vitality	-0.2	0.04	**<0.001**	-0.1	0.05	**0.041**
Mental health	-0.3	0.01	**<0.001**	-0.1	0.1	**0.045**
Social functioning	-0.1	0.04	**<0.001**	-0.02	0.04	0.5
Bodily pain	-0.1	0.01	**0.003**	-0.04	0.04	0.3
General health	-0.2	0.04	**<0.001**	-0.1	0.04	**0.005**

3.1.13 Biological predictors of stress scores

Finally, the biological predictors of stress scores during IFN-α treatment are presented in Table 3.41. After adjusting for baseline stress scores, there was a significant effect of the difference of cortisol values from awakening, at 60 minutes after awakening, in predicting stress scores during treatment (Coefficient=0.4, R^2=0.68, p=0.039).

There was also a significant negative effect of kynurenic acid levels in predicting subsequent stress scores (Coefficient=-1.3, p=0.014) however; this effect was no longer significant after adjusting for baseline stress scores (p=0.2) indicating that this association was driven by baseline stress scores.

There were no significant effects of any of the PUFAs measured. However, after adjusting for baseline stress scores there was a significant negative effect of the omega-3 PUFA alpha-linolenic acid (ALA) (Coefficient=-5.7, R^2=0.43, p=0.034).

Table 3.41 Biological predictors of stress scores during IFN-α treatment

	Post baseline PSS scores			Post baseline PSS scores adjusting for baseline PSS scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Awakening AUCi	0.02	0.01	0.1	0.01	0.01	0.4
Delta 15 minutes	0.2	0.6	0.7	-0.04	0.3	0.9
Delta 30 minutes	0.6	0.5	0.2	0.2	0.3	0.5
Delta 60 minutes	0.8	0.4	0.1	0.4	0.2	**0.039**
Day AUC	-<0.01	<0.01	0.4	-<0.01	<0.01	0.1
Noon	-0.1	0.9	0.9	-0.1	0.5	0.8
8PM	0.6	1.0	0.6	-0.8	0.6	0.2
Tryptophan	0.2	0.5	0.8	0.1	0.4	0.7
Kynurenine	-0.01	0.01	0.4	<-0.01	0.01	0.6
3-Hydroxykynurenine	-0.3	0.4	0.4	-0.2	0.3	0.6
Kynurenic acid	-1.3	0.5	**0.014**	-0.5	0.5	0.2
Kynurenine/Tryptophan	-0.1	0.2	0.4	-0.1	0.1	0.5
EPA	0.3	2.9	0.9	-0.9	2.0	0.6
DHA	0.7	1.4	0.6	-0.2	1.0	0.9
ALA	-4.3	3.9	0.3	-5.7	2.7	**0.034**
AA	-1.0	0.8	0.2	-0.3	0.6	0.7
LA	-0.3	0.3	0.2	-0.1	0.2	0.5
AA/DHA + EPA	-0.6	0.6	0.3	0.2	0.4	0.6

3.1.14 Clinical predictors of anxiety scores

The socio-demographic predictors of anxiety scores during IFN-α treatment are presented in Table 3.42. There was a significant effect of having a previous history of MDD (Coefficient=2.9, p=0.010) however, this was no longer significant after adjusting for baseline anxiety scores (p=0.1) indicating that this effect is driven by baseline anxiety scores. There were no other significant socio-demographic predictors.

The predictive effects of psychosocial stressors are presented in Table 3.43. There were no significant predictive effects of psychosocial stressors on subsequent anxiety scores.

Table 3.42 Socio-demographic predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Age	-0.1	0.05	0.3	-0.04	0.04	0.3
Gender	-1.5	1.3	0.3	-1.4	1.1	0.2
Ethnicity	-0.8	1.2	0.5	0.4	1.0	0.7
Education Level	-0.7	1.7	0.7	0.03	1.4	1.0
Employment	-0.9	1.2	0.5	-1.9	1.0	0.1
Relationship Status	1.1	1.1	0.4	0.5	1.0	0.6
History of MDD	2.9	1.1	**0.010**	1.8	1.0	0.1
Family History	2.4	1.3	0.1	1.3	1.2	0.3
Substance Use	-0.8	1.2	0.5	-1.2	1.0	0.2
Genotype	0.6	0.7	0.4	0.2	0.6	0.8
Viral Load	-0.3	0.2	0.2	-0.2	0.2	0.3
Fibroscan	<0.01	0.1	1.0	-0.01	0.1	0.9

Table 3.43 Psychosocial stress predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Any BLE	1.6	1.1	0.2	1.1	1.0	0.2
Parental Separation	-1.3	1.3	0.3	-0.9	1.1	0.4
Parental Loss	-0.7	1.9	0.7	-1.7	1.6	0.3
Childhood Physical Abuse	1.3	1.6	0.4	1.4	1.4	0.3
Childhood Sexual Abuse	-0.1	1.5	0.9	0.1	1.3	0.9
Any Childhood Trauma	-0.8	1.2	0.5	-0.7	1.0	0.5

The cognitive predictors (illness perceptions) are presented in Table 3.44. There were significant effects of the timeline, consequences, timeline cyclical, personal control and emotional representations dimensions in predicting anxiety scores (Coefficient=0.3, p<0.001; Coefficient=0.3, p<0.001; Coefficient=0.5, p<0.001; Coefficient=-0.5, p<0.001 and Coefficient=0.3, p<0.001, respectively). All of these remained significant after adjusting for baseline anxiety scores (R^2=0.27, p=0.043; R^2=0.31, p=0.005; R^2=0.35, p=0.002; R^2=0.32, p=0.014 and R^2=0.27, p=0.012, respectively) indicating that these effects are independent of baseline anxiety levels.

Table 3.44 Cognitive predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Timeline	0.3	0.1	**<0.001**	0.2	0.1	**0.043**
Consequences	0.3	0.1	**<0.001**	0.2	0.1	**0.005**
Timeline Cyclical	0.5	0.1	**<0.001**	0.4	0.1	**0.002**
Personal Control	-0.5	0.1	**<0.001**	-0.3	0.1	**0.014**
Treatment Control	-0.1	0.1	0.7	0.1	0.1	0.5
Illness Coherence	-0.1	0.1	0.3	0.1	0.1	0.4
Emotional Representations	0.3	0.1	**<0.001**	0.2	0.1	**0.012**

The predictive effects of baseline psychopathology are presented in Table 3.45. There were significant effects of baseline depression, fatigue, stress and anxiety scores on subsequent anxiety scores during IFN-α treatment (Coefficient=0.3, p<0.001; Coefficient=0.4, p<0.001; Coefficient=0.3, p<0.001 and Coefficient=0.6, p<0.001, respectively). After adjusting for baseline anxiety scores, there was still a significant effect of baseline depression scores (Coefficient=0.2, R^2=0.35, p<0.001).

There were significant effects of baseline scores of all 8 health status dimensions on subsequent anxiety scores during IFN-α treatment. These data are presented in Table 3.46. Furthermore, after adjusting for baseline anxiety scores, there were still significant negative effects of baseline scores on the physical functioning, vitality, mental health, social functioning, bodily pain and general health dimensions (Coefficient=-0.1, R^2=0.34, p=0.002; Coefficient=-0.1, R^2=0.35, p=0.003; Coefficient=-0.1, R^2=0.33, p=0.015; Coefficient=-0.1, R^2=0.37, p=0.001; Coefficient=-0.1, R^2=0.35, p=0.002 and Coefficient=-0.1, R^2=0.41, p<0.001, respectively). The effects of physical role limitation and emotional role limitation were no longer significant (p=0.1 and p=0.1, respectively), indicating that the effect of these two dimension were driven by baseline anxiety scores whereas the others are independent of baseline anxiety scores.

Table 3.45 Baseline psychopathology predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
IDS	0.3	0.04	**<0.001**	0.2	0.1	**0.001**
CFQ	0.4	0.1	**<0.001**	0.1	0.1	0.3
PSS	0.3	0.1	**<0.001**	0.2	0.1	0.058
HADS-A	0.6	0.1	**<0.001**	-	-	-

Table 3.46 Baseline health status predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coef.	**Std. Error**	**P Value**	**Coef.**	**Std. Error**	**P Value**
Physical functioning	-0.1	0.04	**0.019**	-0.1	0.03	**0.002**
Physical role limitation	-0.03	0.01	**0.047**	-0.02	0.01	0.1
Emotional role limitation	-0.04	0.01	**0.005**	-0.02	0.01	0.1
Vitality	-0.1	0.02	**<0.001**	-0.1	0.02	**0.003**
Mental health	-0.1	0.03	**<0.001**	-0.1	0.04	**0.015**
Social functioning	-0.1	0.02	**<0.001**	-0.1	0.02	**0.001**
Bodily pain	-0.1	0.02	**0.003**	-0.1	0.02	**0.002**
General health	-0.1	0.02	**<0.001**	-0.1	0.02	**<0.001**

3.1.15 Biological predictors of anxiety scores

The biological predictors of anxiety scores during IFN-α treatment are presented in Table 3.47. There was a significant predictive effect of the difference from awakening, of cortisol values at 60 minutes after awakening (Coefficient=0.5, p=0.004). However, this effect was no longer significant after adjusting for baseline anxiety scores (p=0.1) indicating that this association is driven by baseline anxiety scores.

There were no significant predictive effects of any of the kynurenine and tryptophan pathway metabolites.

There were no significant predictive effects of any of the PUFAs measured on subsequent anxiety scores.

Table 3.47 Biological predictors of anxiety scores during IFN-α treatment

	Post baseline HADS-A scores			Post baseline HADS-A scores adjusting for baseline HADS-A scores		
	Coeff.	**Std. Error**	**P Value**	**Coeff.**	**Std. Error**	**P Value**
Awakening AUCi	0.01	0.01	0.1	<-0.01	<0.01	0.2
Delta 15 minutes	0.3	0.3	0.3	0.2	0.2	0.2
Delta 30 minutes	0.3	0.2	0.2	0.1	0.2	0.7
Delta 60 minutes	0.5	0.2	**0.004**	0.3	0.1	0.1
Day AUC	-<0.01	<0.01	0.3	-<0.01	<0.01	0.2
Noon	-0.02	0.4	1.0	0.03	0.3	0.9
8PM	0.2	0.5	0.8	-0.02	0.4	1.0
Tryptophan	-0.1	0.3	0.9	0.1	0.2	0.8
Kynurenine	-0.01	0.01	0.1	-0.01	0.01	0.1
3-Hydroxykynurenine	0.01	0.2	1.0	<0.01	0.2	1.0
Kynurenic acid	-0.4	0.3	0.1	-0.1	0.2	0.7
Kynurenine/Tryptophan	-0.1	0.1	0.3	-0.1	0.1	0.1
EPA	-0.03	1.4	1.0	-0.2	1.2	0.8
DHA	0.1	0.7	0.8	-0.1	0.6	0.8
ALA	-1.8	1.9	0.4	1.0	1.8	0.6
AA	-0.1	0.4	0.9	-0.2	0.3	0.5
LA	-0.1	0.1	0.4	-0.1	0.1	0.5
AA/DHA + EPA	-0.1	0.3	0.6	-0.1	0.3	0.8

3.2 Qualitative study on nursing staff

Interviewees from all three sites were asked to describe the standard care processes for providing care for hepatitis C patients. Four key themes were identified and are demonstrated below.

3.2.1 Assessing patient risk factors

The participants discussed factors that they identify when assessing the risk of patients developing depression (at baseline) and when assessing any subsequent development of symptoms during the course of IFN-α treatment. The same factors were identified by staff as being relevant when assessing both the risk for depression at baseline, and the development of depression throughout the treatment course. These have been divided into two categories, psychological and other, and are presented in Table 3.48 and Table 3.49, respectively.

Table 3.48 Psychological risk factors assessed by clinical nurse specialists (*n*=9)

Risk Factors	***n***
History/diagnosis (verbal)	
Mental health history	9
Use of medications/anti-depressants	8
Seen by a psychiatrist and/or mental health team	7
Mental health history of family/relatives	4
Previous self-harm	2
Signs and symptoms (verbal)	
Depression	8
Low mood	6
Emotional changes and deterioration	6
Angry/irritable/aggressive	5
Description of ‘how they are feeling’	5
Suicidal thoughts	3
Signs and symptoms (non-verbal)	
Eye contact	6
Tearful	5
Changes in behaviour	4
Agitation/fidgety	3
General demeanour	3

Table 3.49 Other risk factors assessed by clinical nurse specialists (*n*=9)

Risk Factors	***n***
Family/social support	8
Sleep pattern/deprivation	7
Alcohol/drug abuse	6
Employment	6
Housing arrangement	4
Life events (e.g. bereavement)	4
Medical history	4
Weight/diet/appetite	4
Medical co-morbidities	4
Activities/social life	2
Relationship/marital status	2
Fatigue/low energy	1

3.2.2 Co-ordinating action

Further actions clinical nurse specialists take when risks are recognised either prior to or during treatment were also assessed. Four types of actions were identified: involvement of patients and/or relatives, referral to liaison psychiatrists, referral to other resources (community services/GPs) and discussion with nursing staff.

First, the staff emphasised that the decision to refer a patient to a psychiatrist or other mental health professional has to be made jointly with patients, as their engagement is the key to successful treatment. Concerns raised by patients' relatives can also initiate the referral process.

> *"We say that it's not just a one way street, you might come to me and say well I really would like to be referred so it's not always me who says well we would like you to see somebody."*

> *"We do have relatives who phone us and obviously if they talk to us then we will listen, we might not necessarily comment, but we'll then act on it nonetheless."*

Second, in the trusts where liaison psychiatrists are available, their advice is often sought prior to making any formal referrals.

> *"we do work very closely with our liaison consultant psychiatrist, and so if we do feel that we need further advice and we're not quite sure about something, then we will always have an open discussion with him."*

> *"I'd rather contact the psychiatrist and explain what the symptoms are, what the situation is and then he will decide and he will advise us on what to do, how to deal with it."*

Third, clinical nurse specialists can contact a community mental health team and/or patients' GP to co-ordinate their actions. This can either be as an

alternative to, or in conjunction with liaison psychiatry involvement, depending on existing patient involvement in such services as well as the availability of liaison psychiatry services.

> *"If I think someone is going to need some support during treatment, I will get them referred to the community mental health team."*
>
> *"We do sometimes refer patients to the counselling psychotherapy department or recommend that they go to their GP if they are feeling quite low."*

Additionally, informal discussions about patients take place within the nursing team, when nurses perceive a risk regarding patients' well-being. This is on a patient-by-patient basis and serves not only as an information exchange but also as a resource for making decisions.

> *"We also talk about patients a lot if for example, someone's come in and there's been some difficulty, we'll either email everybody to say can you keep an eye on this patient because at clinic they weren't quite right…"*
>
> *"We will also go round and discuss patients within the team "what did you pick up on?" and quite a lot of interesting things come out of that, where we use each other as a resource quite a lot."*

3.2.3 Sources of uncertainty and available strategies to reduce them

Almost all the staff noted that, based on many years of experience, they were confident in their ability to detect signs of psychological deterioration in patients. However, several sources of uncertainty still exist for clinical nurse specialists when making decisions regarding patient welfare. These include familiarity with patient, language barriers and being able to distinguish psychological symptoms from others, as demonstrated in Table 3.50.

Table 3.50 Main sources of uncertainty identified by clinical nurse specialists

Source	Characteristic response
Patient engagement and familiarity	"I think if it's the first time you've met a patient, its actually quite difficult for me to gauge those things." "I keep encouraging patients to not keep it in. If there are problems they need to tell us so we can do something about it rather than just sort of suffer quietly."
Distinguishing psychological symptoms from others	"Sometimes it's difficult to separate out which is a physical things and which is related to their psychological health but I think they're probably all related." "It's hard to know what's sort of a physical symptom from treatment or what is actually psychological…"
Knowing when/whether to refer to psychiatrist	"There's always those more borderline cases where you're not quite sure; should I refer or shouldn't I." "I feel that somebody may benefit from input from the likes of Dr. XXX (liaison psychiatrist), and yet the individual may not feel that, so that could be a little bit more of a difficult one where I've actually gone back and discussed with other staff and said if there were actually concerns."
Language barriers	"also, we're never quite sure if what we want to say is translated to the patient and also if what the patient wants to say to us is translated and I get exactly what they want to say." "some of them will speak English to a limited degree and when you're chatting to them, after a while, once you've heard yes yes yes so many times, you suddenly realise that, or you ask them a question and they give you the wrong answer, I will ask them a question, expecting and knowing what the answer should be and they'll give me the wrong answer and I'll know that they're not understanding."

Developing a good rapport with patients was repeatedly mentioned by nurse specialists as one of the key aspects in the care process, and the key strategy to reduce uncertainty.

> *''I used to have my own patients in a Monday clinic and I knew them really well and I would know instantly if something was wrong. Now we kind of rotate through the clinics so if you don't know someone, it might take a couple of times before you can pick up that they're not quite themselves.''*

Another uncertainty reduction strategy is good communication among team members and also with the wider multidisciplinary group. Despite all the above-mentioned sources of uncertainty, staff felt that they are generally well supported by their own nursing staff, liaison psychiatrists and other community teams. This close-knit team allows them to use each other as a source of information to reduce uncertainty about making decisions on the appropriate course of action.

> *''We've got a good relationship between the different doctors and within the team as well so that's probably helping as well. Supporting each other is very important in these kind of case scenarios because it's difficult to deal with someone who's becoming very unwell and knowing that I've got the support from doctors and the other nurses, can make it easier as well.''*

> *''We're all in the same office and there's quite a good relationship, rapport between us all. I mean, if there's anything that you've seen, you know, Mr so and so, and you know he seems a bit wound up or emotional, we do tend to sort of tell each other what our observations are.''*

3.2.4 Suggested areas of improvement

Interviewees made suggestions for improving their current practice and care processes, including extra time with patients, staff training in mental health, additional support (appointment of liaison psychiatrists and dual qualified nurses, in particular) and the development of a protocol for referrals to psychiatric services.

> *‘‘. . .discussing things with the consultant psychiatrist, which we do, but, it would be nice to have a little more time to do that and to kind of probably set up a formal meeting with him monthly or something that would be very useful.’’*

> *‘‘I’ve often wondered if we had a nurse who was dual qualified so had a mental health qualification as well as a general one, whether that would be of any benefit.’’*

However, the staff emphasised that they feel confident to overcome these issues, and expressed pride in the way they deliver holistic care for patients through management of psychological, physical and social needs. They were asked whether a decision-making support tool would enhance the accuracy of clinical judgements. Although most respondents agreed that such a tool could be utilised as a back-up for their mostly intuitive ways of assessing risks, or as an effective communication aid when different health care professionals are involved in care processes, they emphasised the importance of their own clinical judgement based on knowledge of the patients.

> *‘‘You’ve always got your own gut instinct, that you will rely on and perhaps you would use a tool as a backup really just to confirm what your hunch is.’’*

> *‘‘I would not solely rely on a tool. I would also communicate with other people about the findings. Yeah, I think it needs both, it’s important to communicate your finding and your feeling about it.’’*

4 Discussion

4.1 Summary of findings

This is the first study to extensively investigate the cognitive, biological and psychosocial predictors of IFN-α-induced depression in patients with chronic HCV infection. The main findings in relation to the original aims of this thesis are reported below:

The first primary aim of this thesis was to monitor the impact of IFN-α treatment on a number of clinical and biological. I predicted that:

- IFN-α would lead to an increase in depression, fatigue, stress and anxiety scores.
- IFN-α would lead to a decrease in health status and well-being measures.
- IFN-α would lead to a decrease in tryptophan levels, with a subsequent increase in kynurenine and its neurotoxic metabolites, and a decrease in the levels of the neuroprotective metabolite; kynurenic acid.
- IFN-α would lead to a decrease in the level of omega-3 PUFAs and an increase in omega-6 contents.
- IFN-α would lead to a number of gene expression changes particularly increased expression of genes involved in: tryptophan metabolism, PUFA metabolism and inflammation, and reduced expression of genes involved in GR functionality and neuroplasticity.

Indeed, consistent with a multitude of studies, IFN-α treatment led to an increase in depression, fatigue, stress and anxiety scores. This is accompanied by worsening of health status and decreased well-being. IFN-α treatment also led to changes in a number of the biological systems and metabolites

investigated. Specifically, there is a decrease in tryptophan and kynurenic acid levels accompanied by an increase in kynurenine levels. There is also a decrease in the omega-3 PUFA docosahexaenoic acid (DHA) and in the omega-6 PUFA arachidonic acid (AA). However, there are no significant changes in cortisol levels as a result of IFN-α treatment, although the smaller number of patients with these data may have affected the findings. IFN-α treatment also led to changes in the expression of genes involved in 5 different pathways, as well as several candidate genes.

The second primary aim of this thesis was to identify novel clinical predictors of IFN-α-induced depression and assess the contribution of a number of clinical and lifestyle factors on the subsequent development of IFN-α-induced depression. I predicted that:

- A previous history of depression, a family history of psychiatric illness and baseline psychopathology would be associated with the development of IFN-α-induced depression.
- Exposure to recent stressful life events as well as childhood trauma (physical and sexual abuse, parental loss or parental separation) would be associated with the development of IFN-α-induced depression.
- Negative illness perceptions would be associated with IFN-α-induced depression.

Indeed, I find evidence for a predictive effect of a number of clinical and demographic factors on increasing depression scores during IFN-α treatment. In particular, baseline psychopathology (depression, fatigue, stress and anxiety scores) and negative illness perceptions at baseline predict higher subsequent

depression scores. However, there is no contribution of family history of psychiatric illness, psychosocial stressors, education level or relationship status.

The third primary aim of this thesis was to investigate the contribution of specific biological systems to the development of IFN-α-induced depression. I predicted that:

- Increased cortisol awakening response as well as increased cortisol during the day would be associated with IFN-α-induced depression.
- Lower levels of tryptophan and higher kynurenine metabolite contents would be associated with the development of IFN-α-induced depression.
- Lower omega-3 PUFAs and higher omega-6 PUFAs would be associated with the development of IFN-α-induced depression.
- Increased expression of genes involved in tryptophan metabolism, PUFA metabolism and inflammation accompanied by reduced expression of genes involved in GR functionality and neuroplasticity would be associated with associated with IFN-α-induced depression.

Indeed, I provide evidence that some biological variables predict higher depression scores during IFN-α treatment, independent of baseline depression scores. These include lower baseline cortisol levels during the day and lower baseline kynurenic acid levels. Interestingly, there is also an effect of lower levels of the (theoretically pro-inflammatory) omega-6 PUFA arachidonic acid (AA) on subsequent depression scores. Furthermore, at baseline, there are a number of gene expression differences associated with IFN-α-induced depression. These genes are involved in domains important for depression such as tryptophan metabolism, PUFA metabolism, inflammation, and

neuroplasticity. Moreover, there is also differential expression of IL-28β which has been previously shown to be associated with treatment response. Finally, pathway analysis also shows differential expression of genes belonging to MAPK and neurotrophin signalling pathways.

The final aim of this thesis was to gain an in-depth understanding of staff experiences of, and attitudes towards the identification and monitoring of IFN-α-induced-depression, and their decision-making processes. Specifically, I wanted to investigate the following questions:

- What factors do clinical nurse specialists see as important in determining risk of developing depression, at initial consultation and in on-going monitoring?
- What are the sources of uncertainty for clinical nurse specialists and available reduction strategies?

This qualitative component of the study demonstrates that clinical nurse specialists assess a variety of risk factors for depression development during IFN-α treatment, using a range of information and cues. This informs their decisions on the type of action to take with regards to patients developing IFN-α-induced depression. This part of the study also highlights areas for improvement such as: staff training in mental health, additional support, and the development of clinical guidelines, as suggested by the clinical nurse specialists themselves.

Additional exploratory analyses were also conducted to understand if the clinical and biological predictors identified were specific for the development of depression, or if they also predicted the development of other symptoms. Indeed, aspects of negative illness perceptions such as perceived consequences and emotional representations are also predictive of subsequent fatigue, stress and anxiety scores. Baseline depression is predictive of both subsequent stress and anxiety scores. In keeping with the findings for depression, higher baseline cortisol levels during the day and kynurenic acid levels are also predictive of lower subsequent fatigue scores. Interestingly, I also find that higher baseline levels of the omega-3 PUFA alpha-linolenic acid (ALA) is predictive of lower subsequent stress scores.

4.2 Development of depression and other neuropsychiatric effects during IFN-α treatment

IFN-α led to an increase in depression scores with 40% of the sample developing clinically significant depression. This incidence is consistent with the findings from most prospective studies in which the occurrence of depression is 20-45% (Asnis and De La Garza, 2006, Capuron and Miller, 2004, Raison et al., 2005b). I also find a significant increase in fatigue, stress and anxiety scores during IFN-α treatment. Indeed, depression is not a single symptom but rather a syndrome comprised of emotional, cognitive and neurovegetative abnormalities. In dimensional analyses, it has been previously observed that neurovegetative symptoms, such as fatigue, occur early in IFN-α treatment and tend to persist, whereas depression-specific symptoms develop significantly later in treatment (Capuron and Miller, 2004). Indeed, my findings are consistent with this as I find that fatigue scores increase rapidly, early in IFN-α treatment, and persist. In fact, using the Chalder Fatigue Questionnaire cut-off point of >18, by treatment week 4, 20 individuals (42%) are already fatigued and this rate remains between 20 and 40 (42-83% of the total sample) throughout the treatment course. On the other hand, there is a cumulative increase in the development of depression as illustrated in Figure 3.33. In my study, I also observe a decline in health status and well-being which also occurs early on in treatment and persists. General health status and well-being as well as other measures of quality of life have not been extensively investigated in IFN-α patients before and therefore provide valuable insights in to the broad range of side-effects that are experienced by these patients.

4.3 Biological changes during IFN-α treatment

IFN-α led to changes in a number of the biological systems and metabolites measured. Specifically, there is a decrease in the area under the curve of the increase (AUCi) of the cortisol awakening response and an increase in the area under the curve (AUC) for cortisol during the day, at treatment week 24 when compared to baseline. Unfortunately, these effects were not significant and this is likely due to a lack of statistical power. However, these findings are in line with previous studies. It has been demonstrated that there is hyper-reactivity of the HPA axis in response to acute cytokine administration (Capuron et al., 2003b). However, chronic cytokine exposure is associated with a flattening of the diurnal cortisol curve and increased evening cortisol concentrations, which in turn, are associated with adverse behavioural effects such as depression and fatigue (Capuron and Miller, 2011, Raison et al., 2010a). It must be noted that in my sample there is not a clear cortisol awakening response. This may be due to methodological limitations such as the use of salivettes as opposed to cotton swabs which are able to absorb a larger volume of saliva. It is also possible that HCV infection itself can have an effect on endocrine function (Antonelli et al., 2009), which in turn may impact on the cortisol awakening response. Indeed, studies have shown that in chronic medical illnesses such as cardiovascular disease and cancer, there is a flattening of the diurnal cortisol curve and increased evening cortisol concentrations (Matthews et al., 2006, Sephton et al., 2000)

I also find a reduction in tryptophan and kynurenic acid levels and an increase in kynurenine, 3-hydroxykynurenine and the kynurenine/tryptophan ratio, in all patients over the course of IFN-α treatment. This is in line with several studies

which have also shown reductions in tryptophan and increases in kynurenine levels, as well as levels of quinolinic acid as a result of IFN-α (Bonaccorso et al., 2002b, Raison et al., 2010b, Wichers et al., 2005).

With regards to changes in the levels of PUFAs during IFN-α treatment, I find a significant decrease in the levels of the omega-3 fatty acid docosahexaenoic acid (DHA). Additionally, I also find a decrease in the levels of the omega-6 fatty acid arachidonic acid (AA). This is in contrast to my hypothesis that a reduction in omega-3 PUFA levels would be accompanied by an increase in omega-6 contents. Only one other study has investigated IFN-α-induced changes in PUFA levels, with a specific focus on genetic polymorphisms in two key enzymes of PUFA metabolism (Su et al., 2010). The authors demonstrate “at risk” genotypes to be associated with lower levels of DHA and eicosapentaenoic acid (EPA) during IFN-α treatment. However, the authors do not comment on IFN-α related changes in PUFA levels in all patients. Finally, changes in gene expression as a result of IFN-α treatment will be discussed later.

4.4 Clinical predictors of depression during IFN-α treatment

4.4.1 Baseline psychopathology

The most replicated clinical predictor for developing depression during IFN-α treatment is the presence of mood or anxiety symptoms prior to treatment (Lotrich et al., 2007, Raison et al., 2005a). Indeed, the results from this study support this. Dimensions of baseline psychopathology (fatigue, stress and anxiety scores) significantly predict subsequent depression scores, but after adjusting for baseline depression scores these effects are no longer significant.

In other words, patients with more severe baseline fatigue, stress and anxiety have higher depression scores during treatment, but this is mediated by their high baseline depression scores. However, it must be noted that the baseline assessment was conducted on the first day of IFN-α treatment; as such the emotional impact of starting treatment may have had an effect on baseline psychopathology.

4.4.2 Baseline health status

For the first time in this study, the effect of baseline health status and well-being on subsequent depression scores is also investigated. Emotional role limitation, worse social functioning and having more bodily pain at baseline are all predictive of higher depression scores during IFN-α treatment, independent of baseline depression scores. Taken together, these data suggest that it is not only baseline psychopathology but also baseline health status and quality of life that can predict the development of depression during IFN-α treatment.

4.4.3 Cognitive predictors

This is the first study to investigate the role of illness perceptions on the development of depressive symptoms during IFN-α treatment. Although there are no significant differences in illness perception scores between patients with and without a diagnosis of IFN-α-induced depression, there are significant predictive effects of illness perceptions on increasing depression scores during treatment. Specifically, negative beliefs about timeline that is, believing your illness will last a long time, predict higher depression scores. This is in line with previous studies conducted in other populations also showing aspects of illness perceptions such as strong perceived consequences to be predictive of poorer

health outcomes (Bijsterbosch et al., 2009, Foster et al., 2008, Scharloo et al., 2000). These findings are of clinical importance as, unlike other factors such as socio-demographic variables, these links between beliefs and behaviour provide considerable potential for developing preventative cognitive based interventions. Identifying negative thinking associated with subsequent depression development could help to develop an intervention geared towards fostering more adaptive models and expectations, in order to improve outcomes.

4.4.4 Psychosocial stressors

This is also the first study to investigate the role of psychosocial stressors on the development of IFN-α-induced depression. There is evidence linking exposure to stress, such as childhood trauma, with inflammation and depression in adulthood (Archer et al., 2012, Caspi et al., 2003, Danese et al., 2009, Nanni et al., 2012). However, to date no studies have investigated this relationship in patients undergoing IFN-α treatment. In my study, when looking at the effects of early life stress, there is no significant difference in reports of childhood traumatic events between patients with and without IFN-α-induced depression, or a predictive effect of childhood trauma on depression scores during IFN-α treatment. This lack of association between exposure to childhood trauma and IFN-α-induced depression may be due to the fact that in my sample there is already a much higher prevalence of childhood trauma than in the general population. A recent UK survey of young adults stated that 16% of respondents experienced some form of childhood maltreatment (May-Chahal and Cawson, 2005). In my sample, the prevalence of childhood trauma is more than twice this at, 40%. Furthermore, due to the small sample size, when

investigating the individual types of childhood trauma there are very small numbers. For example, only two patients with IFN-α-induced depression and five patients without IFN-α-induced depression reported experiencing physical abuse in childhood. So the lack of association found between childhood trauma sub-types and subsequent depression scores may be due to decreased statistical power. Finally, there is no predictive effect of exposure to stressful life events in the 6 months prior to treatment on subsequent depression scores. This may seem to be in contrast to the abovementioned findings for baseline perceived stress; however, these measures assess different types of stressors over different time frames. Moreover, although not statistically significant, patients who develop IFN-α-induced depression do have a higher prevalence of experiencing at least one stressful life event in the 6 months prior to starting treatment, when compared with patient who do not develop IFN-α-induced depression (57.9% vs. 37.9%). As such, this lack of association may again be due to decreased statistical power.

4.4.5 Other predictors

Other potential, but less frequently replicated clinical predictors, include a past history of MDD or a family history of psychiatric illness, as well as being female (Raison et al., 2005a, Raison et al., 2005b, Sockalingam and Abbey, 2009). In my study, patients developing IFN-α-induced depression have a significantly higher prevalence of a previous history of MDD when compared to patients who do not develop IFN-α-induced depression (52% vs. 24%). Indeed, of the 17 patients reporting a previous history of MDD, 10 develop IFN-α-induced depression, compared with 9 out of 31 who did not have such history. However, a history of MDD does not have any significant predictive effect on subsequent

depression scores during IFN-α treatment, indicating that history of MDD before starting IFN-α therapy does not unequivocally predict the occurrence of IFN-α-induced depression. Rates of IFN-α-induced depression are not significantly different between females and males or in those individuals with a family history of psychiatric illness. A few studies have also previously reported low academic level to be predictive of developing IFN-α-induced depression (Castellvi et al., 2009, Su et al., 2010). The results of my study do not support this as there are no significant differences in education level between patients with and without IFN-α-induced depression and no predictive effect of education level on depression scores during treatment. However, I do find a significant difference in employment status between the two groups, with higher rates of unemployment in those who develop IFN-α-induced depression. This is in line with the findings of a previous study in which unemployment was associated with an increased risk of new-onset depression during IFN-α treatment (Evon et al., 2009).

4.5 Biological predictors of depression during IFN-α treatment

4.5.1 HPA axis

In this study, I identify some predictive effects of baseline cortisol levels on subsequent depression. Specifically, I find higher cortisol levels at noon as well as a larger AUC for cortisol during the day to be predictive of lower depression scores. This is in apparent contrast to previous studies which found increased cortisol output in the day to be associated with IFN-α-induced increases in depression (Raison et al., 2008, Raison et al., 2010a). However, the findings of these studies assessed cortisol output in the day during IFN-α, not the

association between baseline cortisol and subsequent depression development as I have done. Although not significant, at baseline, patients with a diagnosis of IFN-α-induced depression appear to already have a higher difference of cortisol from awakening at 60 minutes after awakening when compared to patients who do not develop IFN-α-induced depression. Most previous studies have assessed the change in HPA axis function as a result of IFN-α rather than investigating baseline cortisol as a predictor for subsequent depression development. One study has shown that an exaggerated cortisol response to the first injection of IFN-α is a risk factor for developing IFN-α-induced depression. (Capuron et al., 2003b).

4.5.2 Kynurenine and tryptophan pathway

There is a significant predictive effect of baseline levels of kynurenic acid on subsequent depression scores during IFN-α treatment. Specifically, a higher baseline level of kynurenic acid is predictive of lower subsequent depression scores, indeed confirming the notion that kynurenic acid may have a protective role against IFN-α-induced depression. Kynurenic acid is an N-methyl-D-aspartate (NMDA) receptor antagonist generally considered to be neuroprotective, and studies have shown depressed patients to have lower levels of kynurenic acid when compared to healthy controls (Myint et al., 2007). In IFN-α treated patients, several studies have investigated the effects of IFN-α on tryptophan metabolism and changes in kynurenine pathway metabolites (Bonaccorso et al., 2002b, Raison et al., 2010b, Wichers et al., 2005). However, this is the first study to assess the predictive effects of baseline levels of kynurenine and tryptophan pathway metabolites on the development of depression during IFN-α. My findings provide evidence for the potential use of

kynurenine pathway metabolites, in this case particularly kynurenic acid, as biomarkers for early detection of IFN-α-induced depression.

4.5.3 Polyunsaturated fatty acids (PUFAs)

I find a significant effect of higher baseline levels of the omega-6 PUFA, AA in predicting lower levels of subsequent depression during IFN-α treatment. This is in contrast to my hypothesis that higher levels of omega-6 fatty acids would be associated with more depressive symptoms, because of their presumed pro-inflammatory action, and that higher levels of the anti-inflammatory omega-3 fatty acids would be protective against depression. Few studies have previously investigated the role of PUFAs in IFN-α treated patients. One study has shown higher baseline levels of the omega-3 fatty acid DHA to be protective against the development of depression during IFN-α treatment (Su et al., 2010). A second study has shown an elevated ratio of omega-6 to omega-3 PUFAs to predict depression during IFN-α treatment (Lotrich et al., 2012). My findings do not support either of these studies and this may be due to some methodological differences. For example, the study by Su et al. was conducted in a Chinese population and so there may be some ethnic variances as well as cultural difference with respect to diet, when compared to my sample. Furthermore, Su et al. conducted PUFA analysis using erythrocytes whereas I measured PUFA levels in plasma.

4.5.4 Gene expression

4.5.4.1 Hypothesis-free approach investigating baseline gene expression differences

I investigated the role of gene expression changes in IFN-α-induced depression firstly using a hypothesis-free approach. Using a criteria of an absolute fold change of 1.4 and a p-value cut-off of $p<0.005$, I find 8 differentially expressed genes at baseline, in patients who later develop IFN-α-induced depression when compared to patients who do not develop IFN-α-induced depression. Of interest, there is lower expression of Glutathione S-transferase mu 4 (GSTM4) at baseline in patients who develop IFN-α-induced depression when compared to patients who do not develop IFN-α-induced depression. GSTM4 plays an important role in detoxifying various toxicants such as reactive oxygen species which are known to induce oxidative stress (Franco et al., 2007). This suggests that oxidative stress may be involved in the development of IFN-α-induced depression. Indeed, there is evidence that clinical depression and animal models of depression are accompanied by oxidative stress as well as nitrosative stress (Leonard and Maes, 2012, Maes et al., 2011). However, no studies have previously investigated this in IFN-α treated patients.

To date, only three studies have investigated gene expression changes in relation to the development of IFN-α-induced depression (Birerdinc et al., 2012, Felger et al., 2012, Krueger et al., 2011). Only one of these studies specifically investigated differentially expressed genes at baseline which predict the development of IFN-α-induced depression (Birerdinc et al., 2012). The authors report an up-regulation of TNF receptor associated factor-6 (TRAF6) and a down-regulation of transforming growth factor beta-1 (TGF-β1). These two

genes were not within the list of 8 differentially expressed that I find in my sample. However, this may be due to the stringent fold change and p-value criteria that I used. Instead, I have investigated these genes in my hypothesis-driven candidate gene approach.

4.5.4.2 Hypothesis-free approach investigating gene expression changes during IFN-α treatment

Of the other two gene expression studies investigating gene expression changes in relation to the development of IFN-α-induced depression, one study specifically identified genes which are differentially expressed as a result of IFN-α administration (Felger et al., 2012). Specifically, the authors found differential expression of 368 genes at treatment week 12 of IFN-α treatment when compared to HCV patients who are not undergoing treatment. These genes were found to be involved in seventeen pathways including those related to antiviral and inflammatory responses such as interleukin-17 signalling, interferon signalling and communication between innate and adaptive immune cells pathway. In my sample, I also investigated IFN-α-related changes in gene expression and I find a total of 516 genes, belonging to five pathways, to be differentially expressed at treatment week 4 when compared to baseline. Of interest, the “mitogen-activated protein kinase” (MAPK) pathway and the “neurotrophin signalling pathway” were both down-regulated. MDD may also involve an inability of neuronal systems, especially under stress conditions, to show adaptive plasticity, a mechanism known as neuronal plasticity (Pittenger and Duman, 2008). Molecular correlations underlying the mechanisms of the stress response involve the regulation of several neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic

factor (GDNF) and neurotrophin-3 (NT-3), all of which have been previously found to be reduced in MDD patients (Cattaneo et al., 2010, Otsuki et al., 2008, Pandey et al., 2011). Furthermore, it has been demonstrated that the protective function of BDNF is dependent on MAPK, and inhibition of MAPK activity blocks the neuroprotective effects of BDNF (Yang et al., 2012). This down-regulation of BDNF as a result of reduced MAPK activity can also be reversed through the use of antidepressants (Qi et al., 2009). As such, the down-regulation of these two pathways, early on in the course of IFN-α treatment, may be important for the development of depression.

The study by Felger et al. also provides evidence for gene expression changes upon IFN-α administration that are associated with depression development. Specifically, they report an up-regulation of 2'-5'-oligoadenylate synthetase 2 (OAS2), a gene linked to chronic fatigue syndrome, to be differentially expressed in patients with IFN-α-induced depression or fatigue, and to correlate with depression and fatigue scores at treatment week 12. The third previous gene expression study in IFN-α treated patients, also found evidence for gene expression changes upon IFN-α administration that were associated with depression development. Specifically, IL-10 levels were found to be decreased in depressed patients and increased in non-depressed patients at treatment week 4 when compared to baseline (Krueger et al., 2011). Interestingly, the authors also report a non-significant reduction in IL-1β expression in both patient groups at treatment week 4 when compared to baseline.

Based on these findings, I also investigated gene expression changes from baseline to treatment week 4 in patients with and without IFN-α-induced

depression. I find 442 genes to be modulated by IFN-α only in patients who develop IFN-α-induced depression. In contrast only 46 genes were modulated only in patients who did not develop IFN-α-induced depression. Of note, these are predictive changes and are not linked to a presence of depression, as only 4 out of the 19 depressed patients were already depressed at treatment week 4. This indicates that patients, who subsequently develop IFN-α-induced depression, are more biologically sensitive to the effects of IFN-α. Similarly, in a recent gene expression study, MDD patients have been shown to be less sensitive to the effects of dexamethasone when compared to healthy controls, indicating increased GR resistance (Menke et al., 2012).

Pathway analysis of the 442 genes modulated by IFN-α only in patients who develop IFN-α-induced depression, shows these genes to belong to eight pathways. Of interest, the “Janus kinase-signal transducers and activators of transcription” (JAK-STAT) signalling pathway is up-regulated. Acutely, IFN-α binds to type-I IFN receptors expressed on immune cells, activating JAK-STAT and tyrosine kinase signalling, which then increases the expression of cytokines and immunoregulatory genes (Felger et al., 2012). Furthermore, IFN-α-induced activation of JAK-STAT signalling is thought to reduce GR-mediated transcription thus causing impaired GR function (Hu et al., 2009, Pace et al., 2011). “Natural killer cells mediated cell cytotoxicity”, which is an immune response related pathway, is also up-regulated at treatment week 4 when compared to baseline in patients with IFN-α-induced depression. It has been recently reported that the induction of cytotoxic natural killer cell function by IFN-α, correlates with viral response to therapy (Ahlenstiel et al., 2011). An association between activation of this pathway with depression development

during IFN-α treatment has not been previously reported. However, there is evidence indicating an activation of natural killer cell activity, in association with stress and depression development, in co-morbidity with other infectious diseases such as HIV (Cruess et al., 2005). Increased natural killer activity has also been demonstrated in MDD patients following the Trier Social Stress Test (Pace et al., 2006).

Finally, “phosphatidylinositol” (PIP) signalling system and “long term potentiation” (LTP) signalling are both down-regulated at treatment week 4 when compared to baseline in patients who develop IFN-α-induced depression. Both of these signalling pathways are involved in neuronal plasticity, which as mentioned earlier refers to the ability of the nervous system to respond and adapt to environmental challenges (Pittenger and Duman, 2008). However, in a broader sense, neuronal plasticity is intimately linked to cellular responsiveness, and failure of such mechanisms might enhance the susceptibility to environmental challenges, such as stress, and ultimately lead to psychopathology. Indeed, in various brain regions, structural and synapse-related findings seem consistent with a deficit in LTP in depression (Marsden, 2013). Furthermore, there is a wealth of evidence indicating a reduction in various neurotrophic factors in the plasma and serum, and more recently in gene expression of MDD patients (Cattaneo et al., 2010, Otsuki et al., 2008, Pandey et al., 2011). The lower expression of both LTP and PIP signalling that I find at treatment week 4 in patients who subsequently develop IFN-α-induced depression may thus indicate one of the substrates of vulnerability for depression development.

4.5.4.3 Hypothesis-driven candidate gene approach investigating baseline gene expression differences

I also investigated candidate genes belonging to 4 important and interlinked domains: tryptophan metabolism, PUFA metabolism, inflammation, and neuroplasticity. I firstly investigated baseline gene expression differences in patients with and without subsequent IFN-α-induced depression. Patients who develop IFN-α-induced depression have significantly lower expression of indoleamine 2,3-dioxygenase 1 (IDO1) and kynureninase (KYNU) at baseline compared to patients who do not develop IFN-α-induced depression. This is theoretically in contrast to previous findings of increased IDO activity upon IFN-α administration (Bonaccorso et al., 2002b, Wichers et al., 2005). However, these studies did not investigate baseline IDO activity, and also IDO activity is a functional measure while here I am measuring the expression of the gene, all of which may explain the difference in my findings.

Patients who develop IFN-α-induced depression have significantly higher expression of interleukin-28 beta (IL-28β) at baseline when compared to patients without IFN-α-induced depression. Recent genome-wide association studies have specifically identified polymorphisms in the IL-28β gene to be predictive of IFN-α treatment response (Ge et al., 2009). Interestingly, only one study has investigated the association between a polymorphism in the IL-28β gene and side-effects during IFN-α treatment. The authors report the C allele of the rs1297860 polymorphism to be associated with both improved treatment response as well as more somatic complaints (Lotrich et al., 2011). Studies of other IL-28β polymorphisms have also shown treatment response-favourable genotypes to be associated with higher expression of IL-28β (Suppiah et al.,

2009). As such it is possible that higher expression of IL-28β, also as a consequence of these genetic variants, may confer vulnerability to the development of neuropsychiatric effects during IFN-α treatment.

Patients who develop IFN-α-induced depression also have significantly higher expression of interleukin-1 alpha (IL-1α) and interleukin 4 (IL-4) at baseline when compared to patients who do not develop IFN-α-induced depression. IL-1α is a pro-inflammatory cytokine and its expression has been previously shown to be up-regulated in the prefrontal cortex of MDD patients (Shelton et al., 2011). Furthermore, in cells, IL-1α has been shown to inhibit GR function (Wang et al., 2004). Indeed, reduced GR function coupled with high levels of cortisol are indicators of HPA axis dysfunction and are some of the most consistent biological findings in depression (Pariante and Lightman, 2008). It is surprising that I find an increased expression of IL-4 at baseline in patients with subsequent IFN-α-induced depression, as IL-4 is an anti-inflammatory cytokine. However, it is possible that this up-regulation may be in response to the increase in other cytokines such IL-1α. Lastly, patients who develop IFN-α-induced depression have significantly lower expression of TRAF6 at baseline when compared to patients who do not develop IFN-α-induced depression. This is in contrast to the previous study by Birerdinc et al. who reported that pre-treatment up-regulation of TRAF6 predicted the development of depression during IFN-α treatment (Birerdinc et al., 2012).

Patients who develop IFN-α-induced depression have significantly lower expression of NR3C1 (GR) at baseline. Lower expression levels of GR and GR-related molecules in peripheral blood have been previously demonstrated in

MDD patients, particularly in those in a current depressive state (Cattaneo et al., 2012, Fujimoto et al., 2008, Katz et al., 2012). As mentioned, above IFN-α is thought to reduce GR-mediated transcription, thus causing impaired GR function, via the activation of JAK-STAT signalling (Hu et al., 2009, Pace et al., 2011). However, my study is the first to provide evidence indicating there may already be impaired GR function prior to starting IFN-α treatment, and this may confer vulnerability to develop IFN-α-induced depression.

Finally, there were no significant differences between patients with and without IFN-α-induced depression, in their baseline expression of genes related to PUFA metabolism. This may also explain the lack of differences I find in plasma levels of PUFAs between the two groups.

4.5.4.4 Hypothesis-driven candidate gene approach investigating gene expression changes during IFN-α treatment

I then compared changes in the expression of these candidate genes from baseline to treatment week 4 in the two patient groups. Patients without IFN-α-induced depression have a lower expression of indoleamine 2,3-dioxygenase 2 (IDO2) at treatment week 4 when compared to baseline, whereas there is not significant change in patients with IFN-α-induced depression. As such, it is possible that there is a difference in the two groups in their breakdown of tryptophan. There may also be a difference in the subsequent metabolites produced as end products of this breakdown. Specifically, patients with IFN-α-induced depression could have a higher synthesis of quinolinic acid when compared to patients without IFN-α-induced depression. Indeed, IFN-α-induced increases in quinolinic acid have been shown to be associated with increased

depression scores (Raison et al., 2010b). However, quinolinic acid was not measured in my sample and so this association warrants further investigation.

Although there are no differences in the expression of PUFA related genes at baseline between patients with and without IFN-α-induced depression, there are IFN-α related changes in the expression of these genes. Both patients with and without IFN-α-induced depression have significantly higher expression of fatty acid desaturase 2 (FADS2) at treatment week 4 when compared to baseline. FADS2 is an enzyme causing desaturation of fatty acids, and its expression has been previously shown to be elevated in bipolar disorder patients as well as schizophrenia patients (Liu et al., 2009, Liu and McNamara, 2011). However, both patients with and without IFN-α-induced depression also have significantly lower expression of cyclooxygenase 1 (COX1) at treatment week 4 when compared to baseline. COX1 is involved in the conversion of the omega-6 PUFA arachidonic acid (AA) to prostaglandin E2 (PGE2) (Botting, 2006). As such, it is not only involved in PUFA metabolism but also inflammation. It is surprising that COX1 expression is decreased at treatment week 4 when compared to baseline as previous studies have shown an over expression of the other isoform of COX, cyclooxygenase 2 (COX2), in MDD patients when compared to healthy controls (Galecki et al., 2012). Although genetic polymorphisms in the COX2 gene have been previously shown to be associated with IFN-α-induced depression (Su et al., 2010), the expression levels of other COX isoforms have not been previously investigated in these patients and thus require further clarification.

Both patients with and without IFN-α-induced depression have significantly lower expression of interleukin 1 beta (IL-1β), interleukin 1 receptor type 1 (IL-1R1) and interleukin 6 receptor (IL-6R) at treatment week 4 when compared to baseline. Moreover, not only is this down-regulation significant, but the fold changes are also substantial in both patient groups. This is in contrast to previous studies where increased levels of IL-1β and IL-6 have been demonstrated in MDD patients as compared with controls (Dowlati et al., 2010, Howren et al., 2009), and in IFN-α treated patients (Bonaccorso et al., 2002b, Loftis et al., 2008). However, the lower expression of IL-1β is in line with the abovementioned gene expression study by Krueger et al. Although not significant, they also find lower expression IL-1β at treatment week 4 in both depressed and non-depressed patients. Of note, patients with IFN-α-induced depression also have significantly lower expression IL-4 at treatment week 4 when compared to baseline, whereas the expression of IL-4 in patients without IFN-α-induced depression does not change. As mentioned previously, IL-4 is an anti-inflammatory cytokine and so the down-regulation I find only in patients with IFN-α-induced depression suggests that it may be protective against the development of IFN-α-induced depression. Patients with IFN-α-induced depression also have higher expression of interleukin 18 (IL-18) at treatment week 4 when compared to baseline, whereas the expression of IL-18 is unchanged in patients without IFN-α-induced depression. IL-18 is a pro-inflammatory cytokine and has been previously found to be elevated in the plasma and serum of depressed patients (Kokai et al., 2002, Merendino et al., 2002). Furthermore, IL-18 is not only produced by cells of the immune system but it is also found in endocrine tissues such as the adrenal and pituitary glands (Sugama and Conti, 2008). As such, IL-18 may be involved in the

pathophysiology of IFN-α-induced depression via both inflammation and stress pathways. Finally, patients with IFN-α-induced depression also have higher expression of TGFβ-1 at treatment week 4 when compared to baseline, whereas the expression of TGFβ-1 is not significant changed in patients without IFN-α-induced depression. Again, this is in contrast to the findings from Birerdinc et al. who report pre-treatment down-regulation of TGFβ-1 predicted the development of depression during IFN-α (Birerdinc et al., 2012). However, the authors only investigated pre-treatment gene expression whereas I have looked at the change in TGFβ-1 as a result of IFN-α administration.

Patients without IFN-α-induced depression have lower expression of NR3C1 (GR) at treatment week 4 when compared to baseline. This is also surprising as I had hypothesised reduced expression of genes involved in GR functionality to be predictive of IFN-α-induced depression. However, it must be noted that patients who develop IFN-α-induced depression already have lower expression of NR3C1 at baseline when compared to patients who do not develop IFN-α-induced depression. As such, patients without IFN-α-induced depression display a lower expression of NR3C1 at treatment week 4 when compared to baseline; it may be the baseline difference that plays a more important role and confers vulnerability to develop IFN-α-induced depression.

4.6 Clinical predictors of fatigue, stress and anxiety during IFN-α treatment

Although the main focus of this thesis is the identification of factors contributing to the development of depression during IFN-α treatment, I also investigated predictors of other outcomes, such as fatigue, stress and anxiety.

4.6.1 Baseline psychopathology

Interestingly, baseline depression has a significant effect on subsequent stress and anxiety scores even after adjusting for baseline depression scores. This provides further evidence for the importance of baseline psychopathology, and indicates that it does not only confer vulnerability to depression development but also to the development of other symptoms during IFN-α treatment.

4.6.2 Baseline health status

When looking at the effects of baseline health status and well-being, better general health status is predictive of lower fatigue and lower stress scores during IFN-α treatment. Many of the health status and well-being dimensions measured are also predictive of subsequent anxiety scores. Specifically, having more energy, better mental health, better social functioning, less bodily pain and better general health at baseline are all predictive of lower subsequent anxiety scores. These data highlight the importance of not only monitoring health outcomes during and after IFN-α treatment, but actually assessing these prior to commencing treatment in order to identify more vulnerable individuals, not only for depression development but also for the development of other neuropsychiatric symptoms.

4.6.3 Cognitive predictors

There are significant predictive effects of illness perceptions on fatigue, stress and anxiety scores. Specifically, believing your illness to be more serious and with more consequences, and assigning more emotional representations to your illness, are all predictive of higher fatigue scores. Having more emotional representations is also predictive of higher subsequent stress scores. Anxiety scores are also predicted by the timeline, consequences, timeline cyclical, and emotional representations dimensions of the illness perceptions questionnaire. Additionally, there is an effect of the personal control dimension whereby having stronger beliefs about one's own ability to control their symptoms is predictive of lower subsequent anxiety scores. Taken together, this is further evidence to support the notion that identifying negative thinking, and encouraging and improving positive illness beliefs, could provide individuals with more adaptive cognitive models and expectations, and improve outcomes. Indeed, in other populations, interventions aimed at changing illness perceptions have been shown to improve functional health outcomes (Petrie et al., 2002).

4.6.4 Psychosocial stressors

There are no significant effects of psychosocial stressors on stress, fatigue or anxiety scores. However, this may again be due to the higher prevalence of childhood trauma seen in my sample compared to the general population as discussed earlier.

4.6.5 Other predictors

There is a strong predictive effect of a history of MDD on subsequent stress and anxiety scores. There is also a significant predictive effect of ethnicity on

subsequent fatigue scores. Specifically, being from a white British background is predictive of higher fatigue scores during IFN-α treatment. Relatively few studies have examined fatigue levels among different ethnic groups, none in IFN-α patients. Most evidence points towards increased fatigue in minority groups (Jason et al., 1999, Torres-Harding et al., 2008). In my study, the results show the opposite effect; however, this may be due to the fact that the sample is predominantly of a white British background. Furthermore, due to the smaller number of individuals from a variety of different ethnic backgrounds, I dichotomised ethnicity in to white British versus all others. This may also contribute to the opposite results seen in my sample.

4.7 Biological predictors of fatigue, stress and anxiety during IFN-α treatment

4.7.1 HPA axis

A larger increase of cortisol from awakening at 60 minutes after awakening, significantly predicts higher subsequent stress scores. This is again in keeping with the data from Capuron et al. who also found the cortisol response to the first injection of IFN-α to be associated with anxiety symptoms at treatment week 8 (Capuron et al., 2003b). This is further indication that hyper-reactivity of the HPA axis may be a risk factor for IFN-α-induced neuropsychiatric effects. Also in line with the findings for depression, higher baseline cortisol levels at noon as well as a larger AUC for cortisol during the day are also predictive of lower fatigue scores. This is again in contrast to previous studies which have found increased cortisol output in the day to be associated with IFN-α-induced increases in fatigue as well as depression (Raison et al., 2008, Raison et al.,

2010a). However, as outlined above, the study by Raison et al. did not investigate specifically the association between baseline cortisol and the subsequent development of IFN-α-induced symptoms.

4.7.2 Kynurenine and tryptophan pathway

A higher baseline level of kynurenic acid is also predictive of lower subsequent fatigue and stress scores during IFN-α treatment. Further to its actions at receptors, kynurenic acid also has antioxidant properties and can inhibit oxidative stress (Lugo-Huitron et al., 2011). Interestingly, there is emerging evidence to suggest oxidative stress levels contribute to the pathophysiology and clinical symptoms of conditions such as chronic fatigue syndrome (Kennedy et al., 2005, Logan and Wong, 2001). This may explain the association I find between higher levels of kynurenic acid at baseline and lower levels of fatigue. There is also evidence that clinical depression is accompanied by oxidative stress (Leonard and Maes, 2012). Taken together, it is possible that higher baseline levels of kynurenic acid may be involved in the reduction of oxidative stress and consequently be associated with lower levels of depression and fatigue.

4.7.3 Polyunsaturated fatty acids (PUFAs)

A higher baseline level of the omega-3 PUFA alpha-linolenic acid (ALA) is predictive of lower subsequent stress scores. Indeed, in a clinical trial, administration of ALA has been shown to reduce self-reported stress levels as well as cortisol levels in a group of otherwise healthy college students (Yehuda et al., 2005). I do not find any other associations between baseline PUFA levels and fatigue, stress or anxiety scores during IFN-α treatment.

4.7.4 Gene expression

To limit potential false positive findings due to the high number of variables and multiple testing required for the gene expression analyses, I decided to focus only on gene expression in relation to depression.

4.8 Current clinical practice

The qualitative part of this study investigates how clinical nurse specialists currently identify and monitor patient risk factors before and during IFN-α treatment, and make decisions on the appropriate course of action in order to control these risks. The results provide valuable insights into the complex clinical processes that take place and also reveal the multifaceted nature of risk factors staff are presented with and must assess within limited resources. There are two main findings that I will discuss.

Firstly, all clinical nurse specialists employ a combination of verbal and non-verbal cues to gather information about patients' status on all psychological, biological and social dimensions. Staff frequently mention 'eye contact' with patients as a useful indicator, although more subtle and intangible cues including 'changes in behaviour' and 'general demeanour' are also described as important. Recognition of depressive symptoms is therefore made possible partially by building up familiarity and good rapport with patients over time. The importance of the patient-health professional relationship on both medical and psychosocial outcomes is also highlighted by a previous qualitative study (Stewart et al., 2011).

Secondly, certain risk factors, for example, past mental health history, current or past use of medications such as antidepressants, current or past depression and level of available social support are almost unanimously recognised as important. This is in keeping with previous research which has also highlighted the importance of a history of mood disorders (Fontana et al., 2002) and social support (Evon et al., 2011), in vulnerability to IFN-α-induced depression. Interestingly, the importance of social support is also in keeping with previous research based on patients' own experiences of Hepatitis C (Erim et al., 2010, Glacken et al., 2001). Other factors, as highlighted by Table 3.48 and Table 3.49, are also taken into consideration and allow for a holistic approach. However, there is variability among nurses in their use of other information and this may in part be due to the current lack of available guidelines.

In accordance with Naturalistic Decision Making (NDM) studies of experts in other domains, these findings suggest that staff base their judgements and decisions on intuition, drawing on their past experience to recognise patterns and identify risks (Bowers et al., 1990, Klein, 1998). Decisions are not an end point in the process of care, but occur as an on-going process of gathering information, planning and taking action as necessary. Staff see these patterns as subtle and often cannot describe precisely how they gather information or judge a situation as typical. As other studies suggest, intuition can be a highly efficient mode of decision making in limited-resource and time-critical environments (Currey and Botti, 2003, Xiao et al., 1997). However, accounts of adverse events and multiple sources of uncertainty also illuminate the fact that staff recognise the need for further support for enhancing the accuracy of clinical judgements. The lack of guidelines for prioritizing actions in current

clinical practice results in uncertainty and means that nurses rely on a combination of knowledge, intuition, experience and team support.

An additional point this study raises is that organisational and team processes are important in supporting nursing staff's decision making. This is supported by a previous study, which reported significantly greater adherence to antiviral therapy in a clinic based on an integrated mental health and medical approach (Knott et al., 2006). On the other hand, in the two hospitals with liaison psychiatry services, nurses report a tendency to over-rely on liaison psychiatrists, which may exceed the optimal level of referrals. Development of support aids could improve the accuracy of referrals, and therefore the effectiveness of clinical services for hepatitis C patients receiving IFN-α treatment. Finding the balance between precaution and overreliance on psychiatrists is identified by staff as a potential area for improvement. To this end, the team-based decision making in this field merits further research in the future.

4.9 Methodological considerations

There are a few methodological considerations and study limitations that need to be considered in the interpretation of the findings. Regarding the study in IFN-α patients, firstly blood samples were non-fasting and time of blood sampling was not standardised across the sample. This may be of particular importance for the PUFA measurements as they could be affected by diet. However, patients were always seen and assessed at their routine clinic appointments which were either in morning clinics or in afternoon clinics. As such, the blood samples for each patient would have been taken in the same clinic at every time point, therefore minimising some of the potential confounding effects.

Second, I did not collect information about viral illness onset or duration of untreated HCV infection. This may be of particular importance with regards to the illness perceptions findings, as there may be differences between individuals who have had a more recent diagnosis when compared to those who have had a longer duration of illness.

Third, the sample size is relatively small; however, it must be said that this is an extremely difficult group of patients to recruit, and great effort was made to increase recruitment and capture as many patients as possible. Despite increasing the number of study sites, the vast majority of patients who were starting IFN-α treatment were not eligible for this study due to a number of exclusions specified in the study design. For example, in the first year of recruitment, at one site alone, approximately 180 patients were screened and only 20 were eligible and recruited. Further to the exclusion criteria, due to the

fact recruitment took place at teaching hospitals, a large number of patients were also enrolled in to clinical trials using different treatment regimens to the standard combination therapy with IFN-α and ribavirin, as such these patients were also not eligible to take part in this study. Moreover, given the complexity of the assessments, not all participants completed the salivary cortisol collection, therefore further decreasing the power of the statistical analyses for this data. This should be taken into consideration when interpreting the data presented.

Fourth, in this study I did not investigate changes in serum cytokine levels. However, I did conduct a pilot study using two different cytokine multiplex assays (Luminex and Randox) in approximately 15 samples. These analyses showed no significant changes in cytokine levels upon IFN-α treatment and in fact many of the samples were undetected using these assays. As such, I decided to focus on the changes in the gene expression levels of cytokines. For future work, I aim to measure serum cytokine levels based on the most significant gene expression findings from this PhD thesis.

Moreover, a fifth limitation of this study is that the analyses in this thesis were not corrected for multiple testing and therefore there is a potential for increased type 1 error especially given the large number of predictors (around 55) and outcomes (around 30) that were analysed. However, it is important to stress that when investigating the predictive effects, I only focus on 5 major outcomes (depression diagnosis, depression scores, fatigue scores, stress scores and anxiety scores). Microarray gene expression analysis, by its very nature, also includes a large number of variables (individual genes) but strict p-value and

fold change criteria were used, and I only investigated gene expression changes in relation to one outcome variable - depression status. Furthermore, by conducting pathway analysis, the number of variables was reduced into biologically meaningful findings.

A sixth limitation of the study is that the baseline assessment was conducted on the first day of treatment, immediately before the first administration of IFN-α. As such, it is possible that some of the baseline psychopathological ratings may have been affected by patients' concerns and anxieties surrounding starting IFN-α treatment. A baseline assessment that is more remote from the beginning of therapy initiation may be more appropriate.

Finally, there was no control or placebo group used within this study. However, the main aim of this study was not to test the depressive effect of IFN-α, as this is a well-established model, but instead to identify novel predictors for the development of this depression. As such, I used patients who did not develop IFN-α-induced depression as the comparison group against patients who did develop IFN-α-induced depression. Although some placebo control studies have been conducted in the past, it would be unethical to do so now. Instead the effects of IFN-α can be investigated by comparing patients who are undergoing treatment with their own pre-treatment assessments. Furthermore, given that all study assessments (including the collection of blood samples) were conducted alongside routine clinic appointments, it would be quite difficult to obtain the same wealth of data from HCV infected patients who were not undergoing treatment and therefore not attending routine appointments.

With regards to the qualitative study conducted in clinical nurse specialists, limitations include the demographic profile of the three metropolitan, teaching hospitals, which may not be representative of other hospitals. Given that all three hospitals are located in London, the issue of language barriers may be particularly prevalent in this study, and not elsewhere. The relatively small number of participants in this study also raises the question of transferability of our findings to other settings. However, given the focus of this study on a relatively homogeneous professional group with special expertise (Guest et al., 2006), it is likely that even with a small sample the most important factors affecting decision making were identified and that the results are generalisable to similar settings in other locations.

4.10 Integration of the findings and implications for clinical practice and future research

Despite some methodological limitations, this study still provides several lines of evidence for the impact of IFN-α on behaviour and the mechanisms involved. The findings have implications for clinical practice and provide evidence for potential therapeutic targets for treating and preventing depression.

For example, the pathway analysis of my gene expression data provides evidence for the activation of JAK-STAT signalling pathways specifically in patients who develop IFN-α-induced depression. Furthermore, I find a lower expression of GR already at baseline in patients who later develop IFN-α-induced depression when compared to patients who do not. As such, therapies which either block cytokine signalling pathways including JAK-STAT and/or increase GR function could be a point of potential intervention. The higher expression of IL-28β in patients who later develop depression when compared to those who do not could also be of clinical-relevance as a predictive biomarker. Moreover, whilst gene expression analyses may not be clinically practical, IL-28β genotyping is now routinely conducted in patients due to commence IFN-α treatment in order to assess likelihood of treatment response. As such, the association between IL-28β and the development of depressive symptoms warrants further investigation as there is a possibility to implement these findings, at least in this population. Future microarray studies with larger samples are needed to replicate the multitude of gene expression changes found in this study, and to transform these findings in to clinically-relevant biomarkers.

I also find evidence for a predictive effect of baseline kynurenic acid levels on the development of depression. Kynurenic acid levels could be used as a biomarker for the identification of at risk individuals, and aid clinicians in their decision to use antidepressants, or other interventions, prophylactically. Indeed, tryptophan metabolism involves crosstalk between different systems in the periphery and the central nervous system, as such the use of peripheral biomarkers as indirect evidence of central changes for diagnostic and prognostic purposes is not unrealistic. Interestingly, IDO is known to be activated by inflammation and oxidative stress and inhibited by the anti-inflammatory cytokine IL-4. In this study, I show that patients who develop IFN-α-induced depression have decreased expression of GSTM4, which plays an important role in detoxifying reactive oxygen species and therefore potentially in activating IDO. These patients also show decreased expression of IL-4 at treatment week 4 when compared to baseline, which again would lead to increased IDO activation. These findings provide evidence for the involvement and interaction of different biological processes in the development of IFN-α-induced depression. It is important to note that tryptophan metabolism takes place mainly in the liver and so the involvement of this pathway may be of particular relevance to depression development in the context of medical disorders, especially if affecting liver function.

Baseline depression is an important contributor to the development of IFN-α-induced depression. As such, psychotherapeutic strategies or alternative medicine approaches which may serve to reduce stress and anxiety may be a possible preventative strategy in this and other forms of depression in the context of medical disorders. In this study, I also find consistent evidence for a

predictive effect of negative illness perceptions, not only for the development of depression, but also for other neuropsychiatric symptoms. This finding also provides evidence for the potential use of psychotherapeutic strategies within current clinical practice, such as preventative cognitive based interventions, for depression in the context of medical disorders. As this is the first study to investigate the role of illness perceptions in patients receiving IFN-α treatment, of course future studies would need to confirm this association.

Of specific relevance to the clinical management of this population, the findings of this study also demonstrate that a history of MDD, alone does not predict the development of IFN-α-induced depression. This is of particular importance as previously in some cases, treatment has been withheld from patients with a psychiatric history. Moreover, the findings from the qualitative study show a history of psychiatric illness to be consistently considered as a risk factor by clinical nurse specialists. Findings from this and similar studies need to be incorporated into clinical guidelines and in to the training of nurses and clinic staff who are involved in the care of these patients.

4.11 Conclusions

In conclusion, the findings of this study provide evidence for a number of cognitive, psychosocial and biological predictors of IFN-α-induced depression. In particular, negative illness perceptions consistently predict the development of IFN-α-induced depression as well as other neuropsychiatric symptoms. These findings provide a rationale to test the effect of preventative cognitive interventions in these patients. Furthermore, the findings of this study also indicate that lower baseline levels of kynurenic acid could be a biomarker and predictor of IFN-α-induced depression. Finally, the findings of the qualitative study highlight that detection and management of depression in this population is complex, and relies on the availability of clinical experts and good communication within a multidisciplinary team. Together, our observations may prove useful in the future development of clinical guidelines, biomarker tools and preventative interventions.

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Appendix

Social Data Schedule

1) Sex

0	Male	☐
1	Female	

2) Date of birth ☐☐☐☐☐☐

3) Age ☐☐

4) From the list below, how would you describe your ethnicity?

0	White British
1	Mixed
2	Indian
3	Pakistani
4	Bangladeshi
5	Other Asian
6	Black Caribbean
7	Black African
8	Black Other
9	Chinese
10	Other

5) Where did you live for the first 16 years of your life, starting with the place you were born?

Country	City/Town	Street	No. of years
……..|	……..|	……|	…….....
……..|	……..|	……|	…….....
……..|	……..|	……|	…….....
……..|	……..|	……|	…….....
……..|	……..|	……|	…….....

6) With whom do you live now?

0 Alone
1 Alone, with children
2 Partner/Spouse
3 Partner/Spouse and children
4 Parents
5 Other family
6 Friends
7 Other (specify)

…..

7) With whom did you live one year ago?

0 Alone
1 Alone, with children
2 Partner
3 Partner and children
4 Parents
5 Other family
6 Friends
7 Other (specify)

…..

8) With whom did you live five years ago?

0 Alone

1 Alone, with children

2 Partner

3 Partner and children

4 Parents

5 Other family

6 Friends

7 Other (specify)

…….....

9) Since leaving your parents' home, have you ever lived with others?

0 No

1 Yes

10) What is your relationship status now?

0 Single

1 Married/Living with someone

2 In steady relationship

3 Divorced, Separated

4 Widowed

11) What was your relationship status one year ago?

0 Single

1 Married/Living with someone

2 In steady relationship

3 Divorced, Separated

4 Widowed

12) What was your relationship status five years ago?

0 Single

1 Married/Living with someone

2 In steady relationship

3 Divorced, Separated

4 Widowed

13) Have you ever been in a long-term relationship (1 or more years)?

0 No

1 Yes

14) What was the highest level of education you reached?

0 No qualifications

1 GCSE/O' levels

2 A' levels

3 Vocational/college (B. Tecs/NVQs etc.)

4 University/Professional Qualifications

15) Are you employed now?

0 No, unemployed

1 No, student

2 Yes, full-time

3 Yes, part-time

16) Were you employed one year ago?

0 No, unemployed

1 No, student

2 Yes, full-time

3 Yes, part-time

17) Were you employed five years ago?

0 No, unemployed

1 No, student

2 Yes, full-time

3 Yes, part-time

18) Have you ever been employed?

0 No

1 Yes

19) What is your first language?

0 English

1 Other (please state) _ _ _ _ _ _ _ _ _

MAJOR DEPRESSIVE EPISODE

(→ MEANS: GO TO THE DIAGNOSTIC BOXES, CIRCLE **NO** IN ALL DIAGNOSTIC BOXES, AND MOVE TO THE NEXT MODULE)

A1	Have you ever been consistently depressed or down, most of the day, nearly every day, for the past two weeks?	NO	YES
A2	In the past two weeks, have you been less interested in most things or less able to enjoy things that you used to enjoy most of the time?	NO	YES
	IS **A1** or **A2** CODED **YES?** →	NO	YES
A3	**Over the past two weeks, when you have depressed or uninterested:**		
a.	Was your appetite decreased or increased nearly every day? Did your weight decrease or increase without trying intentionally (i.e., by ±5% of body weight or ±8 Ibs. or ±3.5: kgs., for a 160 lb./70 kg. person in a month)?	NO	YES
b.	Did you have trouble sleeping nearly every night (difficulty falling asleep, waking up in the middle of the night, early morning wakening or sleeping excessively)?	NO	YES
c.	Did you talk or move more slowly than normal or were you fidgety, restless or having trouble sitting still almost every day?	NO	YES
d.	Did you feel tired or without energy almost every day?	NO	YES
e.	Did you feel worthless or guilty almost every day?	NO	YES
f.	Did you have difficulty concentrating or making decisions almost every day?	NO	YES
g.	Did you repeatedly consider hurting yourself, feel suicidal, or wish that you were dead?	NO	YES

A4 ARE **3** OR MORE **A3** ANSWERS CODED **YES**? (OR **4 A3** ANSWERS IF **A1** OR **A2** ARE CODED **NO**)?

NO	**YES**
MAJOR DEPRESSVE EPISODE CURRENT	

IF PATIENT MEETS CRITERIA FOR MAJOR DEPRESSIVE EPISODE CURRENT: →

A5	During your lifetime, did you have other periods of two weeks or more when you felt depressed or uninterested in most things, and had most of the problems we just talked about?	NO	YES
b.	Was there an interval of at least 2 months without depression/loss of interest between your current episode and your last episode of depression?	NO	YES

IS **A5b** CODED **YES**?

NO	**YES**
MAJOR DEPRESSVE EPISODE PAST	

MAJOR DEPRESSIVE EPISODE WITH MELANCHOLIC FEATURES

(→MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE **NO**, AND MOVE TO THE NEXT MODULE)

IF THE PATIENT CODES POSITIVE FOR A CURRENT DEPRESSIVE EPISODE (**A4 = YES**), EXPLORE THE FOLLOWING:

A6	a. IS A2 CODED YES?	NO	YES
	b. During the most severe period of the current depressive episode, did you lose your ability to respond to things that previously gave you pleasure, or cheered you up?	NO →	YES

IF NO: when something good happens, does it fail to make you feel better, even temporarily?

IS EITHER **A6a** or **A6b** CODED **YES**?		NO	YES
A7	**Over the past two week period, when you felt depressed and uninterested:**		
a.	Did you feel depressed in a way that is different from the kind of feeling you experience when someone close to you dies?	NO	YES
b.	Did you feel regularly worse in the morning, almost every day?	NO	YES
c.	Did you wake up at least 2 hours before the usual time of awakening and have difficulty getting back to sleep almost every day?	NO	YES
d.	IS A3c CODED YES (RETARDATION OR AGITATION)?	NO	YES
e.	IS A3a CODED YES (ANOREXIA OR WEIGHT LOSS)?	NO	YES
f.	Did you feel excessive guilt or guilt out of proportion to the reality of the situation?	NO	YES

ARE **3** OR MORE **A7** ANSWERS CODED **YES**?

NO	**YES**
Current Major Depressive Episode* with *Melancholic Features	

B. DYSTHYMIA

(→ MEANS: GO TO THE DIAGNOSTIC BOX, CIRCLE **NO**, AND MOVE TO THE NEXT MODULE)

IF PATIENT'S CURRENTLY MEET CRITERIA FOR MAJOR DEPRESSIVE EPISODE, DO NOT EXPLORE THIS MODULE.

B1	Was this period interrupted by your feeling OK for two months or more? →	NO	YES
B3	**During this period of feeling depressed most of the time:**		
	a. Did your appetite change significantly?	NO	YES
	b. Did you have trouble sleeping or sleep excessively?	NO	YES
	c. Did you feel tired or without energy?	NO	YES
	d. Did you lose your self-confidence?	NO	YES
	e. Did you have trouble concentrating or making decisions?	NO	YES
	f. Did you feel hopeless?	NO	YES
	ARE **2** OR MORE **B3** ANSWERS CODED **YES**? →	NO	YES
B4	Did the symptoms of depression cause you significant distress or impair your ability to function at work, socially, or in some other important way? →	NO	YES

IS **B4** CODED **YES**?

NO	**YES**
DYSTHYMIA	
CURRENT	

C. SUICIDALITY

In the past month did you:

C1	Think that you would be better off dead or wish you were dead?	NO	YES
C2	Want to harm yourself?	NO	YES
C3	Think about suicide?	NO	YES
C4	Have a suicide plan?	NO	YES
C5	Attempt suicide?	NO	YES
	In your lifetime:		
C6	Did you ever make a suicide attempt?	NO	YES

IS AT LEAST **1** OF THE ABOVE CODED **YES**?
IF YES, SPECIFY THE LEVEL OF SUICIDE RISK AS FOLLOWS:

C1 or **C2** or **C6** = **YES**: Low

C3 or **(C2 + C6)** = **YES**: Moderate

C4 or **C5** or **(C3 + C6)** = **YES**: High

NO	**YES**
SUICIDE RISK	
CURRENT	

FIGS

Include Grandparents, parents, siblings and offspring aged 18 or above

Keep in mind all those in your family as I go through this following list of questions

Did anyone:

a) Feel very low for a couple of weeks or more, or have a diagnosis of depression?

YES / NO If YES, who? _____

b) Attempt or complete suicide?

YES / NO If YES, who? _____

c) Seem overexcited (or manic) day and night, or have a diagnosis of mania?

YES / NO If YES, who? _____

d) Have visions, hear voices, or have beliefs that seem strange or unreal?

YES /NO If YES, who? _____

e) Have unusual or bizarre behavior, or have a diagnosis of schizophrenia?

YES / NO If YES, who? _____

f) Was anyone hospitalized for psychiatric problems?

YES / NO If YES, who? _____

CHILDHOOD EXPERIENCES

[Selected items from Childhood Experiences of
Care & Abuse-Questionnaire (CECA-Q)]

1) WHO BROUGHT YOU UP BEFORE AGE 17?

Write below the PARENT FIGURES who brought you up in childhood. List each family arrangement with different types of parent figures which lasted a year or longer. Consider natural parents, step-parents (including parents' live in partners), aunt, friends of the family, adoptive parents, foster parents, etc.

If you have only lived in one arrangement, then fill in the first family arrangement and leave the other boxes blank. For example, if this was with your natural parents, write in 'Mother' and 'Father' and age '0'.

Family arrangement	Mother figure	Father figure	Your age at start
First (ALL)			

If you have lived in other arrangements such as with mother alone or mother and step-father, then list them below together with age you were when the arrangement began.

Family arrangement	Mother figure	Father figure	Your age at start
Second (If applicable)			
Third (If applicable)			

Were you ever in a children's home or institution prior to age 17?

0 No
1 Yes

If NO, go to question 2.

If yes: Type of institution e.g. local authority care; hospital, etc.	Age entered	Age left

2) PARENTAL LOSS AND SEPARATION [Please circle or write in answer]

	Mother	Father
Did either parent die before you were aged 17?	No Yes	No Yes
If YES, what age were you?	Age	Age .....
Have you ever been separated from either parent for 6 months or more before age 17?	No Yes	No Yes

If NO separation, then go to question 3.

If YES separated:

At what age were you first separated?	Age	Age .....
How long was this separation for?	..... Years	..... Years
What was the reason for separation?		
Parental illness	No Yes	No Yes
Parental divorce, separation	No Yes	No Yes
Abandoned by parent or never knew parent	No Yes	No Yes
Other reason (please specify below)	No Yes	No Yes

Please describe your experience

.....
.....
.....

3) PHYSICAL PUNISHMENT BEFORE THE AGE OF 17 BY A PARENT FIGURE OR OTHER HOUSEHOLD MEMBER

When you were a child or a teenager were you ever hit repeatedly with an implement such as a belt or stick) or punched, kicked or burnt by someone in the household?

0 No
1 Yes ☐

If NO, go to question 4.

If YES:

	Mother Figure	Father Figure
How old were you when it began?	Age	Age .....
Did the hitting happen on more than one occasion?	No Yes	No Yes
How were you hit?	0 Belt or stick 1 Punched/kicked 2 Hit with hand 3 Other	0 Belt or stick 1 Punched/kicked 2 Hit with hand 3 Other
Were you ever injured, e.g. bruises, black eyes, broken limbs?	No Yes	No Yes
Was this person ever so angry they seemed out of control?	No Yes	No Yes

Please describe your experience
.....
.....
.....

Did you experience this from anyone else in the household?

0 No
1 Yes ☐

Please describe your experience
.....
.....
.....

4) UNWANTED SEXUAL EXPERIENCES BEFORE AGE 17 [Please circle as appropriate]

When you were a child or teenager did you ever have any unwanted sexual experiences?

0 No
1 Yes
2 Unsure

Did anyone force you or persuade you to have sexual intercourse against your wishes before age 17?

0 No
1 Yes
2 Unsure

Can you think of any upsetting sexual experiences before age 17 with a related adult or someone in authority, e.g. teacher?

0 No
1 Yes
2 Unsure

If NONE, end interview.

If YES or UNSURE to any of the above, then complete the following:

	First Experience	Second Experience
How old were you when it began?	Age	Age .....
Was the other person someone you knew?	No Yes	No Yes
Was the other person a relative?	No Yes	No Yes
Did this person do it on more than one occasion?	No Yes	No Yes
Did it involve touching private parts of your body?	No Yes	No Yes
Did it involve sexual intercourse?	No Yes	No Yes

Please describe your experience
.....
.....
.....
.....

Brief Life Events Questionnaire

The following questions are about events or problems which may have happened to you **in the last 6 months** which might have caused you distress and to seek help.

---

1. Did you suffer from a serious illness injury or an assault? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

2. Did a serious illness, injury or assault happen to a close relative? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

3. Did a parent, spouse, partner, child, brother or sister of yours die? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

4. Did a close family friend or other relative die? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

5. Did you have a separation due to marital difficulties or break off a steady relationship? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

5b. Did you end a long lasting friendship with a close friend or relative? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

6. Did you have serious problem with a close friend, neighbour or relative? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

7. Were you made redundant or sacked from your job? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

8. Were you seeking work without success for more than 1 month? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

9. Did you have a major financial crisis such as losing the equivalent of three months income? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

10. Did you have problems with the police involving a court appearance? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

11. Was something you valued lost or stolen? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

12. Did you/your wife or partner give birth to a child? Yes/No Date: _____

If yes, at that time, how bad was that for you? Very Bad ☐ Moderately Bad ☐ Not Too Bad ☐

13. Have you had any other significant negative events?

……..……
…….
When did this happen? ……

Substance Use (Cannabis Experience Questionnaire, Section 2)

Please indicate in the table below any drug(s) (cannabis, amphetamines, cocaine, ecstasy, acid, LSD, tranquilisers, crack, heroin) **including alcohol and tobacco** which you use/have used recreationally, the frequency with which you use/have used this drug, your age when you first tried the drug(s) and whether you are a past or current user. Use a new box for each additional drug: Circle your response(s) as appropriate.

Drug	Frequency	Age	Use	When
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice	Started Stopped	Current Past	Day Night Both day and night

Illness Perception Questionnaire

Your views about your illness

Listed below are a number of symptoms that you may or may not have experienced since your illness. Please indicate by circling yes or no, whether you have experienced any of these symptoms since your illness, and whether you believe that these symptoms are related to your illness.

	I have experienced this symptom since my illness	This symptom is *related to my illness*
Pain	Yes / No	Yes / No
Sore Throat	Yes / No	Yes / No
Nausea	Yes / No	Yes / No
Breathlessness	Yes / No	Yes / No
Weight Loss	Yes / No	Yes / No
Fatigue	Yes / No	Yes / No
Stiff Joints	Yes / No	Yes / No
Sore Eyes	Yes / No	Yes / No
Wheeziness	Yes / No	Yes / No
Headaches	Yes / No	Yes / No
Upset Stomach	Yes / No	Yes / No
Sleep Difficulties	Yes / No	Yes / No
Dizziness	Yes / No	Yes / No
Loss of Strength	Yes / No	Yes / No

We are interested in your own personal views of how you see your current illness.

Please indicate how much you agree or disagree with the following statements about your illness by ticking the appropriate box.

	Views about your Illness	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
IP1 *	My illness will last a short time					
IP2	My illness is likely to be permanent rather than temporary					
IP3	My illness will last for a long time					
IP4 *	This illness will pass quickly					
IP5	I except to have this illness for the rest of my life					
IP6	My illness is a serious condition					
IP7	My illness has major consequences on my life					
IP8 *	My illness does not have much effect on my life					
IP9	My illness strongly affects the way others see me					
IP10	My illness has serious financial consequences					
IP11	My illness causes difficulties for those who are close to me					
IP12	There is a lot which I can do to control my symptoms					
IP13	What I do can determine whether my illness gets better or worse					
IP14	The course of my illness depends on me					
IP15 *	Nothing I do will affect my illness					
IP16	I have the power to influence my illness					
IP17 *	My actions will have no affect on the outcome of my illness					
IP18 *	My illness will improve in time					

	Views about your Illness	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
IP19 *	There is very little that can be done to improve my illness					
IP20	The treatment will be effective in curing my illness					
IP21	The negative effects of my illness can be prevented by my treatment					
IP22	My treatment can control my illness					
IP23 *	There is nothing which can help my condition					
IP24 *	The symptoms of my condition are puzzling to me					
IP25 *	My illness is a mystery to me					
IP26 *	I don't understand my illness					
IP27 *	My illness doesn't make any sense to me					
IP28	I have clear picture or understanding of my condition					
IP29	The symptoms of my illness change a great deal from day to day					
IP30	My symptoms come and go in cycles					
IP31	My illness is very unpredictable					
IP32	I go through cycles in which my illness gets better and worse					
IP33	I get depressed when I think about my illness					
IP34	When I think about my illness I get upset					
IP35	My illness makes me feel angry					
IP36 *	My illness does not worry me					
IP37	Having this illness makes me feel anxious					
IP38	My illness makes me feel afraid					

Causes of my illness

We are interested in what you consider may have been the cause of your illness. As people are very different, there is no correct answer for this question. We are most interested in your own views about the factors that caused your illness rather than what others, including doctors of family, may have suggested to you. Below is a list of possible causes for your illness. Please indicate how much you agree or disagree that they were causes for you by ticking the appropriate box.

	Possible Causes	Strongly Disagree	Disagree	Neither Agree nor Disagree	Agree	Strongly Agree
C1	Stress or worry					
C2	Hereditary – runs in my family					
C3	A germ or virus					
C4	Diet or eating habits					
C5	Chance or bad luck					
C6	Poor medical care in my past					
C7	Pollution in the environment					
C8	My own behaviour					
C9	My mental attitude e.g. thinking about life negatively					
C10	Family problems or worries caused my illness					
C11	Overwork					
C12	My emotional state e.g. feeling down, lonely, empty, anxious					
C13	Ageing					
C14	Alcohol					
C15	Smoking					
C16	Accident or injury					
C17	My personality					
C18	Altered immunity					

In the table below, please list in rank order the three most important factors that you now believe caused your illness. You may use any of the items from the box above, or you may additional ideas of your own.

The most important causes for me: -

1. _____

2. _____

3. _____

Inventory of Depressive Symptomatology (IDS)

Please read each group of statements carefully and then pick out the **one statement** in each group that best describes the way that you have been feeling during the **past seven days**, **including today**. Circle the number beside each statement that you have picked.

1. Falling Asleep

0 I never take longer than 30 minutes to fall asleep.
1 I take at least 30 minutes to fall asleep, less than half the time.
2 I take at least 30 minutes to fall asleep, more than half the time.
3 I take more than 60 minutes to fall asleep, more than half of the time.

2. Sleep During the Night

0 I do not wake up at night.
1 I have restless, light sleep with few brief awakenings each night.
2 I wake up at least once a night, but I go back to sleep easily.
3 I awaken more than once a night and stay awake for 20 minutes or more, more than half the time.

3. Waking up too Early

0 Most of the time, I awaken no more than 30 minutes before I need to get up.
1 More than half the time, I awaken more than 30 minutes before I have to get up.
2 I almost always awaken at least one hour or so before I need to, but I go back to sleep eventually.
3 I awaken at least one hour before I need to, and can't go back to sleep.

4. Sleeping Too Much

0 I sleep no longer than 7-8 hours/night, without napping during the day.
1 I sleep no longer than 10 hours in a 24 hour period including naps.
2 I sleep no longer than 12 hours in a 24 hour period including naps.
3 I sleep longer than 12 hours in a 24 hour period including naps.

5. Feeling Sad

0 I do not feel sad.
1 I feel sad less than half the time.
2 I feel sad more than half the time.
3 I feel sad nearly all of the time.

6. Feeling Irritable

0 I do not feel irritable.
1 I feel irritable less than half the time.
2 I feel irritable more than half the time.
3 I feel extremely irritable nearly all of the time.

7. Feeling Anxious or Tense

0 I do not feel anxious or tense.
1 I feel anxious/tense less than half the time.
2 I feel anxious/tense more than half the time.
3 I feel extremely anxious/tense nearly all of the time.

8. Response of Your Mood to Good Events

0 My mood brightens to a normal level which lasts for several hours.
1 My mood brightens but I do not feel like my normal self when good events occur.
2 My mood brightens only somewhat to a rather limited range of desired events.
3 My mood does not brighten at all, even when very good or desired events occur in my life.

9. Mood in Relation to Time of Day

0 There is no regular relationship between my mood and the time of day.
1 My mood often relates to the time of day because of environmental events (e.g. being alone, working).
2 In general, my mood is more related to the time of day than to environmental events
3 My mood is clearly and predictably better or worse at a particular time each day.

9A. Is your mood typically worse in the morning, afternoon or night (circle one).

9B. Is your mood variation attributed to the environment? Yes / No (circle one).

10. The Quality of your Mood

0 The mood (internal feelings) that I experience is very much a normal good mood.
1 My mood is sad, but this sadness is pretty much like sad mood I would feel if someone close to died or left.
2 My mood is sad, but this sadness has a rather different quality to it than the sadness I'd feel if someone close to me died or left.
3 My mood is sad, but this sadness is different from they type of sadness associated with grief or loss.

Please complete <u>either</u> 11 <u>or</u> 12 (not both)

11. Decreased Appetite

0 There is no change in my usual appetite.
1 I eat somewhat less often or lesser amounts
2 I eat much less than usual and only with personal effort.
3 I rarely eat within a 24hour period, and only with extreme personal effort or when other persuade me to eat.

12. Increased Appetite

0 There is no change in my usual appetite.
1 I feel a need to eat more frequently than usual,.
2 I regularly eat more often and/or greater amounts of food than usual.
3 I feel driven to overeat both at mealtimes and in between meals.

Please complete <u>either</u> 13 <u>or</u> 14 (not both)

13. Decreased Weight (within the last 2 weeks)

0 I have not had a change in my weight.
1 I feel as if I've had a slight weight loss.
2 I have lost 2 pounds or more.
3 I have lost 5 pounds or more.

14. Increased Weight (within the last 2 weeks)

0 I have not had a change in my weight.
1 I feel as if I've had a slight weight gain.
2 I have gained 2 pounds or more
3 I have gained 5 pounds or more

15. Concentration/Decision Making

0 There is no change in my usual capacity to concentrate or make decisions.
1 I occasionally feel indecisive or find that my attention wanders.
2 Most of the time, I struggle to focus my attention or to make decisions.
3 I cannot concentrate well enough to read or cannot make even minor decisions,

16. View of Myself

0 I see myself as equally worthwhile and deserving as other people.
1 I am more self-blaming than usual.
2 I largely believe that I cause problems for others.
3 I think almost constantly about major and minor defects in myself.

17. View of my future

0 I have an optimistic view of my future.
1 I am occasionally pessimistic about my future, but mostly I believe things will get better.
2 I'm pretty certain that my immediate future does not hold much promise of good things for me.
3 I see no hope of anything good happening to me anytime in the future.

18. Thought of Death or Suicide

0 I do not think of suicide or death.
1 I feel that life is empty or wonder if it's worth living.
2 I think of suicide or death several times a week for several minutes.
3 I think of suicide or death several times a day in some detail, or I have made specific plans for suicide or have actually tried to take my life.

19. General Interest

0 There is no change from usual in how interested I am in other people or activities.
1 I notice that I am less interested in people or activities.
2 I find I have interest in only one or more of my formerly pursued activities.
3 I have virtually no interest in formerly pursued activities.

20. Energy Level

0 There is no change in my usual level of energy.
1 I get tired more easily than usual.
2 I have to make a big effort to start or finish my usual daily activities

3 I really cannot carry out most of my usual daily activities because I don't have the energy.

21. Capacity for Pleasure of enjoyment (excluding sex)

0 I enjoy pleasurable activities just as much as usual.
1 I do not feel my usual sense of enjoyment from pleasurable activities.
2 I rarely get a feeling of pleasure from any activity.
3 I am unable to get any pleasure or enjoyment from anything.

.

22. Interest in Sex (please rate <u>interest</u>, not activity)

0 I'm just as interested in sex as usual.
1 My interest in sex is somewhat less than usual or I do not get the same pleasure from sex as I used to.
2 I have little desire for or rarely derive pleasure from sex.
3 I have absolutely no interest in or derive no pleasure from sex.

23. Feeling Slowed Down

0 I think, speak, and move at my usual rate of speed.
1 I find that my thinking is slowed down or my voice sounds dull or flat.
2 It takes me several seconds to respond to most questions and I'm sure my thinking is slowed.
3 I am often unable to respond to questions without extreme effort.

24. Feeling restless

0 I do not feel restless
1 I'm often fidgety, wringing my hands or need to shift how I am sitting.
2 I have impulses to move about and am quite restless.
3 At times, I am unable to stay seated and need to pace around.

25. Aches and Pains

0 I don't have any feeling of heaviness in my arms or legs and don't have any aches or pains.
1 Sometimes I get headaches or pains in my stomach, back or joints but these pains are only sometimes present and they don't stop me from doing what I need to.
2 I have these sorts of pains most of the time.
3 These pains are so bad that they stop what I am doing.

26. Other bodily symptoms

0 I don't have any of these symptoms: heart pounding fast, blurred vision, sweating, hot and cold flashes, chest pain, heart turning over in my chest, ringing in my ears, or shaking
1 I have some of these symptoms but they are mild and are present only sometimes
2 I have several of these symptoms and they bother me quite a bit.
3 I have several of these symptoms and when they occur I have to stop doing whatever I am doing

27. Panic/Phobic Symptoms

0 I have no spells of panic or specific fears/phobia
1 I have mild panic episodes or fears that do not usually change my behaviour or stop me from functioning.
2 I have significant panic episodes or fears that force me to change my behaviour but do not stop me from functioning.
3 I have panic episodes at least once a week or severe fears that stop me from carrying on my daily activities.

28. Constipation/Diarrhoea

0 There is no change in my usual bowel habits.
1 I have intermittent constipation or diarrhoea which is mild.
2 I have diarrhoea or constipation most of the time but it does not interfere with my day to day functioning.
3 I have constipation or diarrhoea for which I take take medicine or which interferes with my day to day functioning.

29. Interpersonal Sensitivity

0 I have not felt easily rejected, slighted, criticised or hurt by others at all.
1 I have occasionally felt rejected, slighted, criticised or hurt by others.
2 I have often felt rejected, slighted, criticised or hurt by others, but these feelings have had only slight effects on my relationships or work.
3 I have often felt rejected, slighted, criticised or hurt by others and these feelings have impaired my relationships or work.

30. Leaden Paralysis/Physical Energy

0 I have not experienced the physical sensation of feeling weighted down and without physical energy.
1 I have occasionally experienced periods of feeling physically weighted down and without physical energy, but without a negative effect on work, school, activity level.
2 I feel physically weighted down and without physical energy, more than half the time.
3 I feel physically weighted down and without physical energy, most of the time, several hours per day, several days per week.

Hospital Anxiety & Depression Scale

Instructions: Read each item and place a firm tick in the box opposite the reply which comes closest to how you have been feeling in the past week. Don't take too long over your replies: your immediate reaction to each item will probably be more accurate.

I feel tense or 'wound up':		**A**	**I feel as if I am slowed down:**	**D**	
Most of the time		3	Nearly all of the time	3	
A lot of the time		2	Very often	2	
Time to time, occasionally		1	Sometimes	1	
Not at all		0	Not at all	0	
I still enjoy the things I used to enjoy:	**D**		**I get a sort of frightened feeling like 'butterflies in the stomach':**		**A**
Definitely as much	0		Not at all		0
Not quite so much	1		Occasionally		1
Only a little	2		Quite often		2
Not at all	3		Very often		3
I get a sort of frightened feeling like something awful is about to happen:		**A**	**I have lost interest in my appearance:**	**D**	
Very definitely and quite badly		3	Definitely	3	
Yes, but not too badly		2	I don't take as much care as I should	2	
A little, but it doesn't worry me		1	I may not take quite as much care	1	
Not at all		0	I take just as much care as ever	0	
I can laugh and see the funny side of things:	**D**		**I feel restless as if I have to be on the move:**		**A**
As much as I always could	0		Very much indeed		3
Not quite so much now	1		Quite a lot		2
Definitely not so much now	2		Not very much		1
Not al all	3		Not at all		0
Worrying thoughts go through my mind:		**A**	**I look forward with enjoyment to things:**	**D**	
A great deal of the time		3	A much as I ever did	0	
A lot of the time		2	Rather less than I used to	1	
From time to time but not too often		1	Definitely less than I used to	2	
Only occasionally		0	Hardly at all	3	
I feel cheerful:	**D**		**I get sudden feelings of panic:**		**A**
Not at all	3		Very often indeed		3
Not often	2		Quite often		2
Sometimes	1		Not very often		1
Most of the time	0		Not at all		0
I can sit at ease and feel relaxed:		**A**	**I can enjoy a good book or radio or TV programme:**	**D**	
Definitely		0	Often	0	
Usually		1	Sometimes	1	
Not often		2	Not often	2	
Not at all		3	Very seldom	3	

Total A:___ Total D:___

Chalder Fatigue Questionnaire

We would like to know more about any problems you have had with feeling tired, weak or lacking in energy in the **LAST MONTH.** Please answer all the questions by ticking the answer which applies to you most closely. Please choose only one option per question.

	Less than usual (0)	No more than usual (1)	More than usual (2)	Much more than usual (3)
Do you have problems with tiredness?				
Do you need to rest more?				
Do you feel sleepy or drowsy?				
Do you have problems starting things?				
Do you start things without difficulty but get weak as you go on?				
Do you lack energy?				
Do you have less strength in your muscles?				
Do you feel weak?				
Do you have difficulty concentrating?				
Do you have problems thinking clearly?				
Do you make slips of the tongue when speaking?				

Perceived Stress Scale- 10 Item

The questions in this scale ask you about your feelings and thoughts during the **last month**. In each case, please indicate with a check how often you felt or thought a certain way.

Please write here the time of the day at which this questionnaire was completed: _____

1. In the last month, how often have you been upset because of something that happened unexpectedly?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

2. In the last month, how often have you felt that you were unable to control the important things in your life?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

3. In the last month, how often have you felt nervous and "stressed"?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

4. In the last month, how often have you felt confident about your ability to handle your personal problems?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

5. In the last month, how often have you felt that things were going your way?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

6. In the last month, how often have you found that you could not cope with all the things that you had to do?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

7. In the last month, how often have you been able to control irritations in your life?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

8. In the last month, how often have you felt that you were on top of things?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

9. In the last month, how often have you been angered because of things that were outside of your control?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
0=never 1=almost never 2=sometimes 3=fairly often 4=very often

Medical Outcomes Study Short-Form 36 (SF-36)

1 In general how would you say your health is?

Excellent	☐
Very Good	☐
Good	☐
Fair	☐
Poor	☐

2 Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago?	☐
Somewhat better now than one year ago?	☐
About the same	☐
Somewhat worse now than one year ago?	☐
Much worse now than one year ago?	☐

3 The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so how much?

	Yes limited a lot	*Yes limited a little*	*No not limited at all*
Vigorous activities such as running, lifting heavy objects, participating in strenuous sports	☐	☐	☐
Moderate activities such as moving a table, pushing a vacuum cleaner, bowling or playing golf	☐	☐	☐
Lifting or carrying groceries	☐	☐	☐
Climbing several flights of stairs	☐	☐	☐
Climbing one flight of stairs	☐	☐	☐
Bending, kneeling or stooping	☐	☐	☐
Walking more than a mile	☐	☐	☐
Walking half a mile	☐	☐	☐
Walking 100 yards	☐	☐	☐
Bathing or dressing yourself	☐	☐	☐

4 During the past 4 weeks have you had any of the following problems with your work or other regular daily activities as result of you physical health?

	Yes	*No*
Cut down the amount of time you spent on work or other activities.	☐	☐
Accomplished less than you would like	☐	☐
Were limited in the kind of work or other activities	☐	☐
Had difficulty performing the work or other activities (for example, it took extra effort)	☐	☐

5 During the past 4 weeks have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	Yes	*No*
Cut down the amount of time you spent on work or other activities.	☐	☐
Accomplished less than you would like	☐	☐
Didn't do work or other activities as carefully as usual	☐	☐

6 During the past 4 weeks to what extent have your physical health or emotional problems interfered with your social activities with family, friends, neighbours or groups?

- Not at all ☐
- Slightly ☐
- Moderately ☐
- Quite a bit ☐
- Extremely ☐

7 How much bodily pain have you had during the past 4 weeks?

- None ☐
- Very mild ☐
- Mild ☐
- Moderate ☐
- Severe ☐
- Very severe ☐

8 During the past 4 weeks how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	☐
A little bit	☐
Moderately	☐
Quite a bit	☐
Extremely	☐

9 How much of the time during the past 4 weeks…

	All of the time	*Most of the time*	*A Good bit of the time*	*Some of the time*	*A little of the time*	*None of the time*
Did you feel full of life?	☐	☐	☐	☐	☐	☐
Have you been a very nervous person?	☐	☐	☐	☐	☐	☐
Have you felt so down in the dumps that nothing could cheer you up?	☐	☐	☐	☐	☐	☐
Have you felt calm and peaceful?	☐	☐	☐	☐	☐	☐
Did you feel worn out?	☐	☐	☐	☐	☐	☐
Have you felt downhearted and low?	☐	☐	☐	☐	☐	☐
Did you have a lot of energy?	☐	☐	☐	☐	☐	☐
Have you been a happy person?	☐	☐	☐	☐	☐	☐
Did you feel tired?	☐	☐	☐	☐	☐	☐
Has your health limited your social activities (like visiting your friends or relatives)?	☐	☐	☐	☐	☐	☐

10 Please choose the answer that best describes how true or false each of the following statements is for you.

	Definitely true	*Mostly true*	*Not sure*	*Mostly false*	*Definitely false*
I seem to get ill more easily than other people	☐	☐	☐	☐	☐
I am as healthy as anybody I know	☐	☐	☐	☐	☐
I expect my health to get worse	☐	☐	☐	☐	☐
My health is excellent	☐	☐	☐	☐	☐

Salivary Cortisol Collection

Wake up (before 10 a.m.). Immediately after waking up collect your saliva putting the Sorbette under the tongue and leaving it for 60 seconds, then put it back in the tube marked 0.

Write here the EXACT TIME OF AWAKENING: _____

Try to sit down and relax in the next hour. **YOU CANNOT BRUSH YOUR TEETH AND CANNOT HAVE ANYTHING TO EAT OR DRINK FOR THE NEXT HOUR.** If you need, you can drink water, but only immediately AFTER you have taken the sample.

15 minutes after waking up, collect your saliva using the tube marked 15.

• What time is it now? _____

• What were you doing before giving the sample?_____
_____

• Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:
_____
_____

• Did you have any difficult or tense situation, unpleasant thoughts or any kind of pain before taking this sample? If yes, please describe it here:_____
_____

30 minutes after waking up, collect your saliva using the tube marked 30.

• What time is it now? _____

• What were you doing before giving the sample?
_____
_____

• Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:
_____
_____

• Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here:_____
_____

60 minutes (1 hour) after waking up collect your saliva using the tube marked 60.

• What time is it now? _____

• What were you doing before giving the sample?

_____

_____

• Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

_____

_____

• Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here:_____

_____

******* You can now have breakfast and brush your teeth! *******

At 12, noon - before lunch collect your saliva using the tube marked 12.

You should not eat or drink anything, or do not brush your teeth in the 30 minutes before noon.

• What time is it now? _____

• What were you doing before giving the sample?

_____

_____

• Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

_____

_____

• Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here:_____

_____

At 8 pm - before dinner collect your saliva using the tube marked 8.

You should not eat or drink anything, or do not brush your teeth in the 30 minutes before 8pm.

• What time is it now? _____

• What were you doing before giving the sample?

_____

_____

• Did you accidentally have anything to eat or drink before taking the sample? If yes, please describe it here:

_____

_____

• Did you have any difficult or tense situation, unpleasant thought or any kind of pain before taking this sample? If yes, please describe it here:_____

_____

Store the tubes away from the heat and direct sunlight and put them into the fridge as soon as possible.

Please note name and time of any medication taken today (including the contraceptive pill):

_____

_____

Do you have any medical problem? If so, please list them here:

_____

_____

If you are female: Please indicate the age of your first menstrual cycle:

_____

And please indicate the date of the first day of your last menstrual cycle:

_____

Qualitative Study Semi-Structured Interview Topic Guide

Please note that these questions may vary depending on the context of the interview and may be modified as the study progresses to take into account emerging results.

Exploration of role

1. Please explain your role
2. How long have you been doing this job?
3. Could you please explain the process for treating Hepatitis C patients?

Assessment of Hepatitis C patients

4. At the assessment interview what aspects of the patients' psychological history do you ask about? Prompt – previous psychiatric hospitalization, previous psychiatric diagnosis, suicidal ideation, family history of psychiatric illness, aggression, alcohol, drug use, previous aggression or violence.
5. What other risk factors are important? Prompt – recent bereavement, current employment, current relationships and social support, financial status, other health problem, current activities
6. At the assessment interview what psychological signs and symptoms do you assess? Prompt – current mood, depression, anxiety, aggression, agitation, suicidal ideation, withdrawal, crying spells, feelings of hopelessness, rumination, guilt, sleep problems, fatigue.
7. At the follow up visits what psychological signs and symptoms do you assess? Prompt – current mood, depression, anxiety, aggression, agitation, suicidal ideation, withdrawal, crying spells, feelings of hopelessness, rumination, guilt, fatigue, sleep problems, fatigue.
8. At the follow up visits what other risk factors do you assess? Prompt – alcohol and drug use, life events, employment, relationships, social support, financial status, other health problems, current activities.
9. Do you use formal assessment instruments at either the initial assessment or the follow up visits? Prompt BDI, HAD, Zung
10. If there is anything else that you think would help your clinical decision making, what would that be? (e.g. formal assessment tool, staff support group, education and training)
11. (Hypothetically speaking, if a decision making support tool is developed) To what extent would you be willing to use such a tool, and how much do you think you would rely on it? (how much do they value other sources of judgements such as their own experience, personal relationship with patients and advice from peers and specialists)
12. What would prompt you to refer a patient to the psychiatrist prior to commencing treatment? Prompt – signs, symptoms, history, life events
13. What do you base your judgement of the urgency on when you decide whether you would refer a patient to the psychiatrist?
14. What would prompt you to refer a patient to the psychiatrist during treatment – signs, symptoms, history, life events
15. How confident are you that the risk of a patient developing psychiatric side effects can be predicted? Can you tell me more about how confident you are in assessing risks?

Adverse experiences

16. Can you remember a serious or unexpected incident occurring with a patient while they were taking the medication? Prompt – suicide, aggression, violence, unexpected response. (If they cannot think of any AIs – can you remember an episode where you were uncertain about your decision? Which aspect were you not certain about? How did you deal with it?)
17. Can you tell me more about it? What were your first impressions of the patient? Were you surprised?
18. Do you think there is a way to identify such patients before a problem occurs?

19. What are the main challenges of your role?
20. What are the rewarding aspects of your role?